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# Water plants in the Gezira canals

## A study of aquatic plants and their control in the canals of the Gezira cotton area (Anglo-Egyptian Sudan)

BY F. W. ANDREWS, *Botanist, Agricultural Research Institute, Department of Agriculture and Forests, Sudan Government*

(With Plate 1 and 8 Text-figures)

The geographical position and climatic conditions of the Gezira cotton area are briefly described. A description is given of the Blue Nile river, and Sennar Reservoir. The common water plants of the latter are listed. It is shown in the case of five of these plants that they tend to be divided in those that occur in shallow water and those that prefer deeper water. Details of the annual rise and fall of the water of the Reservoir are also given.

The system of canalization in the Gezira is described and details given of the method of irrigation. A list is given of the common water plants in the Gezira canals, and their distribution over the canalized area is discussed. A comparison is made of the water flora of the canals of Egypt with those of the Gezira.

A system of small experimental canals was used to study the life history of the more important water plants in the Gezira canals. Details are given of the extent of the spread of the plants in these canals during a period of 14 months. It was found that abundant growth and seed formation occurred when clear water was entering the canals, but that the bulk of the vegetative part of the plants tended to die when turbid flood water arrived. Abundant seed germination occurred during the turbid water period. Drying the canal bed for 3½ months had little effect on the final quantity of weed developed in the canals when they were refilled. Seed remained viable after 3½ months' dry exposure. Removal of the surface soil from the dry canal bed produced no significant control of the water plants when water again entered the canals. A short flush of water was allowed to flow down the dry canal bed. It was hoped that the seed would germinate and the seedlings be killed by exposure after the flush had ceased. The effect of this treatment on the subsequent plant growth was negligible.

Laboratory experiments on the more important water plants showed that the vegetative part of the plants was killed after 8 days' dry exposure to the sun and that mercuric chloride, mercuric chloride-iodide, and sodium arsenite in a concentration of 1/10,000 killed all plants after immersion in the poison solution for 5 days. At a concentration of 1/100,000 and immersion for 7 days none of these poisons was able to kill *Potamogeton nodosus* Poir. Work on poisons was discontinued for reasons stated in the text.

A short account is given of plants that invade the canals from the banks.

The problem of water plant control is discussed, and it is shown that the most hopeful method of control would be a system whereby all infested canals are cleared at regular intervals designed to prevent seeding during the clear-water period and to remove seedlings during the flood-water period. The application of this method in the Hag Abdulla Subdivision is described. The successful control that resulted reduced the weeding costs by half.

### 1. INTRODUCTION

In this paper it is proposed to describe the aquatic plants in the irrigation canals of the Gezira and the measures that have lately been developed to stem their extremely rapid growth and spread.

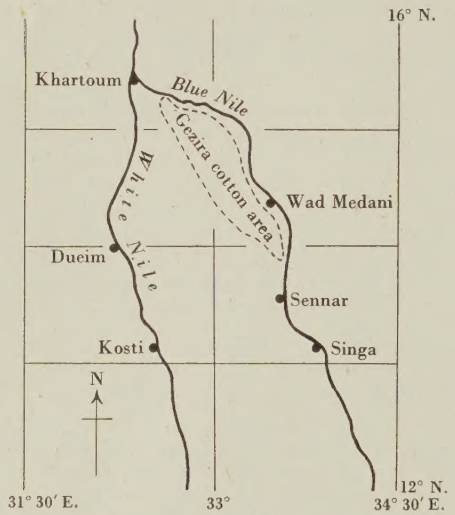
The Gezira cotton area lying on the west side of the Blue Nile river and irrigated by gravitational flow from the Sennar Reservoir extends northwards to about 48 km. south of Khartoum, a total distance of about 225 km. This area is part of the great arid plain that stretches between the Blue and the White Niles and has its apex at Khartoum (Text-fig. 1).

The average maximum and minimum shade temperatures and average rainfall as recorded at the Gezira Research Farm, Wad Medani, for the period 1919–40 are shown in Text-fig. 2. It will be observed that this area in the neighbourhood of Wad Medani receives about 16 in. of rain. Lesser rain occurs to the north and west of the area. The rainfall is mostly concentrated into July, August, and September, and it is during this period that the Blue and White Niles are in flood and abundant silt is brought down the Blue Nile.

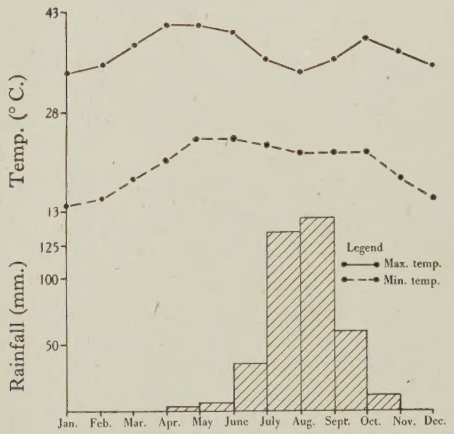
### 2. THE BLUE NILE RIVER, THE SENNAR RESERVOIR, AND THEIR AQUATIC PLANTS

This river as it occurs in the Sudan has a length of about 770 km. to Khartoum where it joins the White

Nile. Garstin (1899) states: 'The average width of the channel throughout its course is 500 metres rarely exceeding 700 metres. The average height of the banks over summer water level is from 8–9 metres for the first 250 kiloms. upstream of Khartoum.




Text-fig. 1. Rough map showing Gezira cotton area. Scale 1: 4,000,000.

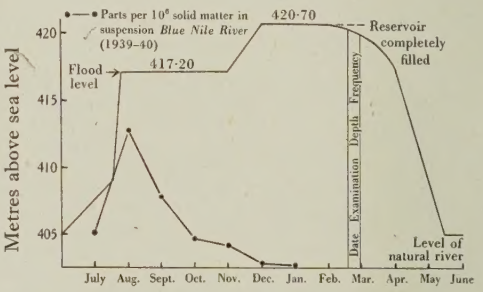



Text-fig. 2.

Further south they average over 10–12 metres above low water level. The difference in level between flood and summer is from 7–8 metres, and in the first quarter of the year the river is reduced to a succession of deep pools connected by very shallow reaches. The Blue Nile is at its lowest in April, the rise in level begins in June and the maximum height is attained in August. In the latter half of September it begins to fall rapidly.'

'In winter the water is very clear having a limpid blue colour. In flood, being charged with the scourings of Abyssinian mountains and forests it is heavily charged with silt and is of a chocolate colour.' It is this silt that has gradually built up the fertile agricultural land of the Nile Delta. Text-fig. 3 shows the average monthly figures, supplied by the Government Analyst, Khartoum, for 1939–40 for solid matter in suspension in the Blue Nile river.

Characteristic of the Blue Nile are the 'Mayas' or mud-flats which are inundated for some months by the river and also by the Sennar dam if they are situated within its influence. These mud-flats occur in the inside of a bend in the river, and their formation is probably due to the rapidly flowing flood water impinging on the outside bank of the curve which it erodes, while silt is deposited and shallow flats formed




Text-fig. 3. Rise and fall of reservoir level during the year.

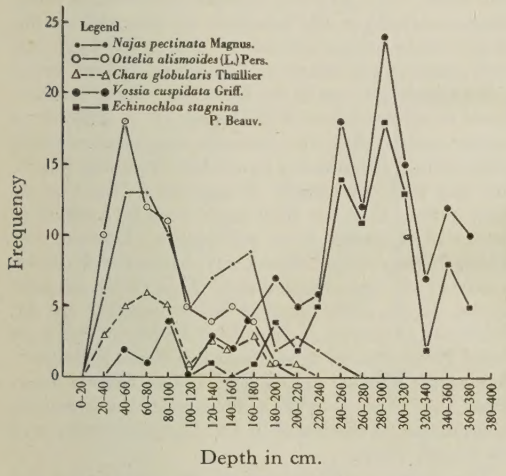
on the inside of the curve as the course of the river at this place moves almost imperceptibly at right angles to the direction of flow. This phenomenon is often found in meandering rapidly flowing silt-carrying rivers (Salisbury, 1907). These shallow banks are inundated for varying periods of time depending on the height and duration of the flood. They are characterized by groves of principally *Acacia arabica* Willd. The water on these mud-flats is comparatively stagnant, and it is there that water plants abound.

The Sennar Reservoir, a shallow artificial lake about 80 km. long and up to 4¼ km. broad, created by the Sennar dam has been colonized by the water plants that occur on the mud-flats of the Blue Nile river. An examination on 19–29 Feb. 1940 of the reservoir near the Sennar dam showed that the flora consisted principally of the following aquatic plants:

*Nymphaea caerulea* Savign.
*Ceratophyllum demersum* Linn.
*Najas pectinata* (Parl.) Magnus
*Ottelia alismoides* (L.) Pers.
*O. ulvifolia* (Planch.) Walp.

*Utricularia thonningii* Sch.
*U. stellaris* Linn.
*Pistia stratiotes* Linn.
*Chara globularis* Thuillier
*Nitella batrachosperma* Agardh.
*Vossia cuspidata* (Roxb.) Griff.
*Echinochloa stagnina* P. Beauv.
*Panicum meyerianum* Nees.
*Juncellus alopecuroides* (Rottb.) C.B.Cl.
*Cyperus rotundus* Linn.
*Polygonum glabrum* Willd.
*P. lanigerum* R.Br.
*Ipomoea reptans* Poir.

These plants are distributed in varying numbers according to the depth of the water. Text-fig. 4 shows the depth-frequency curve of five of the commonest water plants along the banks of this portion of the Reservoir, and around a partially submerged island




Text-fig. 4. Depth frequency in Sennar Reservoir.

which is situated towards its centre. It will be noted that the five plants divide themselves into two different groups: those inhabiting shallow water, viz. *Najas pectinata*, *Ottelia alismoides* and *Chara globularis*, and those which thrive best in deep water, viz. *Vossia cuspidata* and *Echinochloa stagnina*.

The Reservoir water is at its highest level by 1 Dec. and remains so until 1 Feb., when the water is slowly released. By the end of May the Reservoir is empty, the basin almost dry, with the shallow Blue Nile river meandering through it. From mid-May until about mid-July the head reach of the Main Canal is dry, water being pumped up to the scheme from the Blue Nile river for domestic purposes only. From mid-July to October when the river is in flood, the dam is regulated to raise the river level to command the Main Canal. After October, when the silt-laden water becomes clear, the dam is further regulated and the Reservoir refilled. Text-fig. 3 shows the changes in level of the Reservoir throughout the year.

It is evident that during the drying out of the Reservoir from February to July the growth of these water plants receives a severe check. An examination during the middle of April 1940 of the drying land of the Reservoir showed that abundant seed germination of all plants was taking place, but only *Vossia cuspidata* Griff. and *Echinochloa stagnina* P. Beauv. were still surviving vegetatively. *Nymphaea* sp. persisted in wet places. As the water shallows the area becomes overgrown with these two grasses, the more densely covered portions being eagerly eaten by cattle. The rhizomes are pressed into the soil by the hoofs of the cattle and abundant root formation occurs at the nodes. Continuous grazing causes these rhizomes to sprout afresh: thus grazing tends to perpetuate these grasses. On the other hand, the removal of these grasses to permit the annual growing of *Sorghum* and maize on the less densely covered areas tends to rid the Reservoir land of these two plants. When the Reservoir is refilled reinfestation occurs from seed and from rhizomes in the grazed land or that not brought under cultivation. Doubtless some vegetative growth of the plants is preserved in pockets in the river itself, and this would assist in the reinfestation. The survival of these plants is assisted by the rainfall at Sennar. The mean monthly rainfall for the period 1905–29 during May, June, July and August was 16, 59, 121 and 170 mm. respectively.

### 3. The Gezira canals, the canals of Egypt, and their aquatic plants

The Sennar dam and about one-third of the present canalization were completed and in operation by 1925. Since then additional canals have been added (principally to the north and west), and at the present date the scheme, in conformity with the present crop rotation, is irrigating annually nearly 400,000 acres of cropped land, out of a total of 880,000 acres of canalized area.

The canalization system has a total of 2650 miles of canals. A Main Canal receives water (via the Sennar dam) from the Blue Nile river. From the Main Canal, Major canals take off and supply Minor distributaries from which are led off the field outlet channels which irrigate the cropped land.

Irrigation normally commences towards the end of July and continues in the case of cotton, the main economic crop, to the beginning of April. After April many of the canals are left to dry out, those required for domestic purposes, viz. for houses and native villages, being maintained at reduced level. The canals are refilled during the latter part of July.

The method of irrigation is unique in that the Minor distributaries are designed to receive water for 24 hr. but to store the supply at night, as the field channels draw water only during the day. This method of irrigation was introduced to meet certain unusual conditions: it is not intended to perpetuate

it, as it is contrary to sound irrigation practice. During the night the level of water in the Minor distributaries is raised 20–30 cm., and they become, in fact, pools, stagnant except for the rise in level. This night-storage method is known to be advantageous to weed growth. The rate of flow in these canals is very small, the gradient on an average being 7 cm. or less in a length of 1 km. during the day, and nothing at night. The slow rate of flow also provides conditions suitable for water-plant growth. The beds of the canals are roughly semi-elliptic in cross-section. The depth of water varies greatly but rarely exceeds 150 cm., and in most Minor distributaries the water when at full level has no greater depth than 60 cm. It is in the Minor distributaries that water-plant growth is most extensive, and it is with these distributaries that this investigation is principally concerned. These Minor canals are designed to have at the high-water mark a breadth of 8·6 m. Their length varies greatly but averages about 7·5 km., though there are several much longer. The beds and banks of the canals consist of almost impermeable alkaline clay, locally impregnated with sulphates. A comparison of the chemical properties of the water of the Blue Nile river and the irrigation canals has been made by Greene & Snow (1939). They show that the water of the irrigation canals is somewhat more alkaline and in general has a slightly lower calcium/sodium ratio than the Blue Nile river, but there is a small seasonal variation bound up with the river flood in July.

In irrigation reports, weed infestation of the canals was first recorded in 1929. It would appear from the reference that the plant was probably *Potamogeton perfoliatus* Linn. From 1929 onwards there has been a rapid increase in intensity and extent of the water plants in the Gezira canals with a corresponding increase in the annual cost of weed clearance to permit passage of the water. In 1937 it was decided to investigate the problem in order to discover methods for controlling the growth of these plants. Accordingly, during October to December of that year a survey was made of the water plants in all canals. A record was made of plant type, intensity and position in each canal.

The following plants were present in the water, or invading the canals from the banks:

*Aquatic: free floating:*

*Spirogyra decima* (Muell.) Ktz.
*S. maxima* Witr.
*S. crassa* Ktz.

*Aquatic: anchored to the mud:*

*Potamogeton perfoliatus* Linn.
*P. nodosus* Poir.
*P. crispus* Linn.
*P. pectinatus* Linn.
*Chara globularis* Thuillier
*Ottelia alismoides* (L.) Pers.
*Polygonum glabrum* Willd.
*Juncellus alopecuroides* (Rottb.) C.B.Cl.
*Ceratophyllum demersum* Linn.
*Najas pectinata* (Parl.) Magnus
*Nitella batrachosperma* Agardh.
*Vallisneria aethiopica* Fenzl.
*Zannichellia palustris* L. var. *intermedia* Pearsall
*Typha angustifolia* Linn.

*Invading the canals from the banks:*

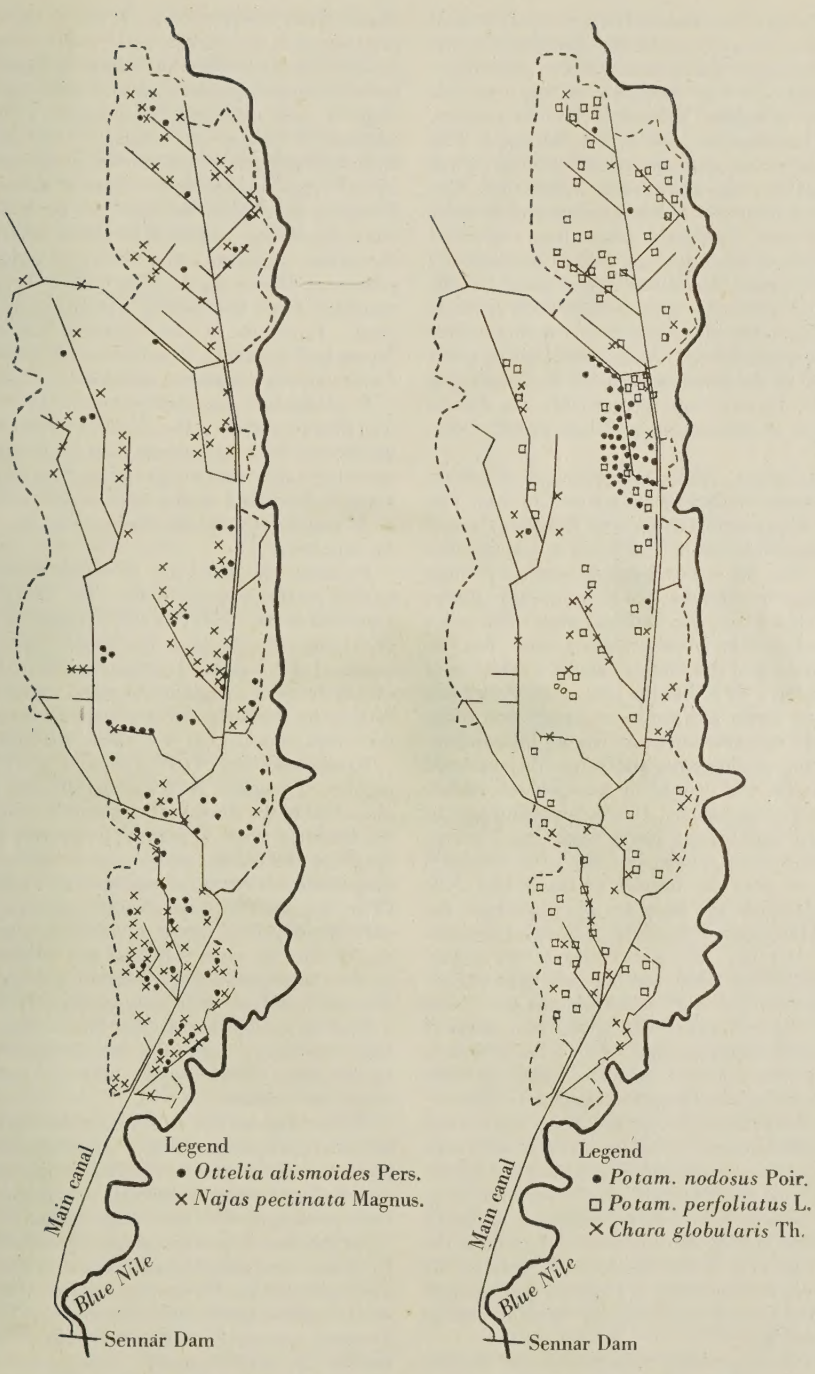
*Panicum meyerianum* Nees.
*P. repens* Linn.
*Echinochloa stagnina* P. Beauv.
*Cyperus rotundus* Linn.
*Alternanthera sessilis* R.Br.
*Ipomoea reptans* Poir.
*Vossia cuspidata* (Roxb.) Griff.
*Phragmites mauritianus* Kunth.

The five common plants of the Sennar Reservoir mentioned (p. 3) occur, as would be expected, in the canals, but it will be noted that some of the water plants occurring in the Reservoir are absent from the canals while others present in the canals have not been found in the Reservoir. That some plants occur in the canals and not in the Reservoir might be attributed to adverse flow of the water. It is difficult to explain satisfactorily the converse, since seed of these plants would presumably be washed down the Reservoir and into the canals. It appears in the case of these plants that the dam itself acts as a physical barrier in retaining seeds and plants. Examination during March of the Main Canal immediately down stream of the dam showed only those plants present in the canals, and none of those peculiar to the Reservoir. Analysis of the soil of the bed of this part of the Reservoir has shown that it differed little from that of the Gezira canals, and it has already been noted that there is little difference in the chemical composition of the water of the Blue Nile river and the Gezira canals.

The distribution of some of the plants over the canalized area is given in Text-fig. 5.

*Najas pectinata* (Parl.) Magnus, *Ottelia alismoides* (L.) Pers. and *Chara globularis* Thuillier (which prefer shallow parts of the Reservoir) were scattered throughout the scheme with a tendency for a concentration in the shallower canals and at the southern end, i.e. nearest the Reservoir.

*Potamogeton perfoliatus* Linn., not found anywhere in the Sudan except in the Gezira canals, shows a general distribution within the irrigated area with concentrations towards the northern and southern ends. This plant, suspected of being present in Hamid en Nil canal in 1929, was definitely referred to as '*Potamogeton* weed' in an irrigation report on the same canal in April 1930. This canal was in operation in 1923 and, since this plant is the only *Potamogeton* sp. present there, it is reasonable to suppose that its presence first became obvious in 1929, and that its introduction to the canal occurred at least a year earlier. *P. perfoliatus* Linn. is essentially a plant of temperate climes, being 'widely dis-




Text-fig. 5. Gezira canalized area showing water-plant distribution. (For clarity minor distributaries and some major canals have been omitted.) Scale 1 : 750,000.

tributed in Europe, northern Africa, temperate Asia and North America and also occurs (perhaps adventively) in Southern India and Eastern Australia' (Dandy, 1937). It was recorded in 1931 by M. Dalleni on Mt Madigue, Tibesti, Tchad, at 1200 m. and by R. Chundeau in 1905 at Tit, Ahaggar. The only specimen of this plant recorded from Egypt was found in 1942 in a seepage pool at Ballana nr. Abu Simbel, a few miles north of Faris, a northern frontier town of the Sudan. The plant has not been recorded from the canals of the Egyptian delta, nor from any other part of Egypt, nor from Africa south of the Gezira canals. It would appear that when suitable conditions arise this plant can thrive in both temperate and tropical climes. Assuming that the plant first appeared in the canalized area in 1928, its rapid spread to the Gezira from presumably far distant sources forms an interesting problem of plant dispersal.

As is well known, Africa forms one of the main routes for aquatic birds migrating from Europe. The north-south migration from Central Europe is one of the most important, but it is difficult to assume that the seed of this plant has been brought by these migrants, since the plant has not been found in Egypt until we reach a place, viz. Ballana, about 600 miles south of the Egyptian Mediterranean coast, nor between this place and the Gezira canals, a distance of about 550 miles. It is hardly conceivable that the migrants have flown a distance of nearly 600 miles without alighting somewhere en route. This migration is said to be a slow one, and it is to be expected that seed on the feet would be knocked off before reaching the Gezira canals. If the migration was in a south-east direction the route might be Tibesti-Gebel Marra-Gezira canals, but then the migrants would have to pass the barrier of the White Nile where the plant has not been found, and where the birds would most certainly alight. Though perennial fresh-water streams and ponds occur on Gebel Marra, *P. perfoliatus* Linn. has not been found there though Lynes collected *P. panormitanus* Biv. from this region. Lynes (1921) also states that there is markedly no migration passage through Gebel Marra or Central Darfur. It is a significant fact that this plant has not yet spread from the canals to the Sennar Reservoir, a distance of only 55 km. from the nearest occurrence of the plant. If the seed is habitually bird carried it would seem inevitable that the plant would have reached the Reservoir. That the seed has been carried the whole way inadvertently on a bird's plumage seems hardly more probable than does the suggestion that one of the British residents in the Gezira was a keen fisherman in England and brought the seed to the Gezira on his fishing boots or among his fishing tackle.

*P. nodosus* Poir. is widely dispersed in the warmer parts of both hemispheres. It has a curiously restricted distribution in the Gezira canals, but is absent from the Reservoir. There is no record of its occurrence in the Sudan until its authoritative identification from the Gezira canals in 1936. It must, however, have appeared several years earlier, for by 1936 it was infesting in quantity a considerable number of canals. *P. crispus* L. not found in the Reservoir and sparsely scattered in the northern part of the canalization was recorded from Kordofan Province in 1837–8 by Kotschy, by Broun in 1905 from the White Nile at Khartoum, and recently by the author from the canal of a small irrigated cotton scheme at Dueim on the White Nile. It has not been recorded from the Sudan section of the Blue Nile river. It occurs in the Egyptian canals, in Lake Nyasa and Southern Rhodesia, so its presence in the Gezira canals causes no comment.

*P. pectinatus* Linn., also not found in the Reservoir, was first recorded in the Sudan in 1936 when it was discovered in a few places in the Gezira canals. It must have appeared much earlier than this date. This plant is described as the most widely spread species of *Potamogeton*, and its presence in the canals is to be expected.

*Juncellus alopecuroides* (Rottb.) C.B.Cl., widely spread in the tropics of the Old World, is widely scattered in the Reservoir and over the canalized area, occurring principally at the water's edge and at the heads of the canals. It prefers shallow water. Just downstream of a regulator a small mound of mud is formed by the rush of water through the regulator: it is on this mound that this plant is frequently found.

*Vessia cuspidata* Griff., occurring in the swamp regions of tropical Africa generally and in India, is abundant in the Reservoir and distributed throughout the irrigated area. It is most frequently found at the heads of the canals, preferring to spread where the deeper water occurs, as was noticed in the Reservoir (Text-fig. 4). The form of this plant appears to vary with the depth of water in which it is growing. On the White Nile river it occurs as a tall stout grass up to 7 ft. high producing a head of 4–8 spikes. In the Sennar Reservoir it is only about 2 ft. high having a head of 2–3 spikes. In the Gezira canals its vegetative portion creeps over the surface of the water, rarely rising about 15 in., with a head of one or rarely two spikes.

*Typha angustifolia* Linn. occurred as a few plants in widely scattered places. It is surprising that this plant was not found in the Reservoir, and that with its wind- and water-dispersed seed it has not become more widespread in the Gezira canals.

Of the less important plants, *Echinochloa stagnina* P. Beauv. was sparsely scattered over the central and southern Gezira, *Polygonum glabrum* Willd. occurred as a few plants in the southernmost part of the scheme, *Ipomoea reptans* Poir. was widespread but only occurred in small groups. *Phragmites mauritianus* Kunth. was only found in two places, as was also *Vallisneria aethiopica* Fenzl. With the exception of

the last named, all these plants were found in the Reservoir.

Comparing the flora of the Gezira canals with that of the canals of Egypt it must be remembered that the water of Egypt is derived from the union at Khartoum of the Blue and White Niles. The water flora of the latter differs from that of the Blue Nile in having a wider range of plants. The following vegetation has been reported by Simpson (1932) as occurring in the canals of Egypt.

*Aquatic: free floating:*

*Eichornia crassipes* Schlecht.
*Pistia stratiotes* Linn.

*Aquatic: anchored to mud:*

*Ceratophyllum demersum* Linn.
*Nymphaea caerulea* Sav.
*N. lotus* Linn.

*Invading from the banks of the canals:*

*Agrostis verticillata* Vill.
*Alternanthera sessilis* R.Br.
(= *A. achyranthoides* Forsks.)
*Cyperus auricomus* Sieb.
*C. articulatus* Linn.
*Diplachne fusca* (L.) Beauv.
*Echinochloa stagnina* P. Beauv.
*Jussieua repens* Linn.
*Paspalidium geminatum* Stapf.
*Paspalum distichum* Linn.
*Phragmites communis* Trin.
*Polygonum senegalense* Meissn.
*P. serrulatum* Lag.
*Juncellus alopecuroides* (Rottb.) C.B.Cl.
*Typha angustata* Bory & Chaub.
*T. latifolia* Linn.

*Eichornia crassipes* Schlecht. is certainly an introduction, due most probably as in other countries to the misplaced enthusiasm of gardeners attracted by its beautiful blue flowers. Legislation has been effected in the Sudan to prevent its introduction into the canals of the Gezira.

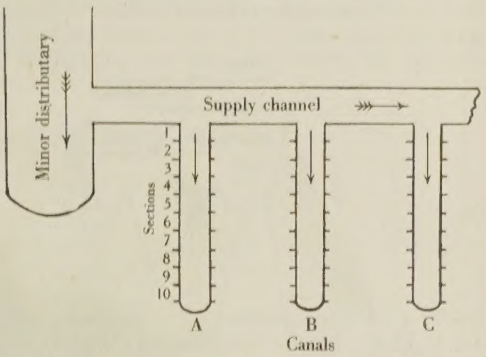
*Pistia stratiotes* Linn. occurs abundantly in the Blue and White Niles and in the Reservoir but appears unable to colonize the Gezira canals. It is a free-floating plant and should not be affected by depth of the water, etc. It can easily be grown in dishes in the laboratory. It is a plant, however, that thrives most in shady places among water grasses and in pools heavily infested with other aquatic plants. The Gezira canals are completely without shade, no trees being allowed to grow on the banks. It is possible, therefore, that this is an important factor in its non-establishment but does not explain its complete absence. The plant collects in abundance at the Sennar dam and has at times caused alarm that it would enter and infest the canals, but no plant has yet been found there.

*Nymphaea* spp., particularly heavy in the White Nile, are possibly unable to become established in the Gezira canals owing to their shallow depth, lack of shade, and the diurnal rise and fall of 20–30 cm. in the level of the water.

All the *Potamogeton* spp. found in the Egyptian canals are present in those of the Gezira.

*Ceratophyllum demersum* Linn. has been found in only one small area in the Gezira canals but is widespread in those of Egypt.

The irrigation system in Egypt differs somewhat from that in the Gezira. There is a continuous flow of water, and night storage with its consequent formation of 'pools' once every 24 hr. is not practised. Except for this, however, the difference would appear to be in design of canal and method of water supply rather than such as would cause major differences in aquatic flora. The Egyptian canals are of considerably greater age than those of the Gezira, and it is conceivable that in the course of years similar conditions of the water type and intensity may prevail in both areas.




Text-fig. 6. Design of experimental canals.

## 4. Observations on aquatic plants in experimental canals

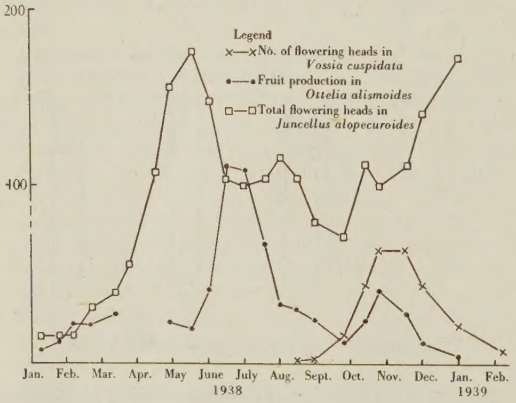
It was clear that a study of the life history of these plants could not be carried out in the canals of the Gezira. From these canals the plants are periodically removed to allow the passage of the water, and during part of the year some of the canals, when not in use, are allowed to become dry. A small canal system was therefore constructed in December 1937 by the Sudan Irrigation Department to be used solely for aquatic plant investigations.

This experimental canal system was situated near the Gezira Research Farm, Wad Medani. Water was obtained from a supply channel taking off from a perennially watered Minor distributary. From this supply channel and at right angles to it were dug a dozen small canals each 50 m. long, and lettered A to L. Each canal was marked off by iron stakes into ten sections numbered 1–10 (Text-fig. 6). The width of each canal at high-water mark was 3·60 m. The water in these canals was stagnant except that

they were occasionally 'topped up' to high-level mark. Each canal, therefore, in miniature represented the Minor distributary at night when the water is practically stagnant except for the rise in level. They provided ideal conditions for water-plant growth. The tail of the supply channel was connected by pipe to a drain so that the whole system could be emptied in one day.

Of the plants present in the Gezira canals the following were considered worthy of immediate attention:

*Potamogeton nodosus* Poir.
*P. perfoliatus* Linn.
*Ottelia alismoides* (L.) Pers.
*Juncellus alopecuroides* (Rottb.) C.B.Cl.
*Chara globularis* Thuillier
*Najas pectinata* (Parl.) Magnus
*Vossia cuspidata* (Roxb.) Griff.




Text-fig. 7. Flowering and fruiting per 3 sections through the year.

The other *Potamogeton* spp. present in the canals, though of great potential menace, were small in extent and could be dealt with summarily.

In January 1938 each section of the experimental canals was sown transversely with one of the above seven water plants employing a distribution such that each canal contained each of the seven plants at least once. The position in the canals was such that each of the seven plants was sown at least once at the head, tail and centre of the canals. Sowing the plants across the canals ensured that each plant had the opportunity to grow in different depths of water.

During the succeeding year the plants were left unmolested and periodic observations were made on the growth, time of flowering and fruiting. Some flowering and fruiting curves are given in Text-fig. 7.

The result of the year's observations may be summarized as follows:

(1) Rapid growth and production of seed of all plants (except *Chara globularis* Thuillier) occurred during the time that clear water was being supplied from the Sennar Reservoir, viz. from January to July and October to January.

(2) When the cloudy flood water arrived in July the bulk of the vegetative part of the plants tended to die down, due presumably to the excessive turbidity of the water reducing the light. This effect has also been noticed in the Deccan canals (Inglis & Gokhale, 1941). Abundant germination of seed occurred during this period. Little vegetative growth of the seedlings was made, but roots and rhizomes were formed in abundance and would be ready for the return of the clear water when vegetative growth would be rapid.

(3) *Potamogeton* spp. produced flowers and fruit during the whole period of clear water: no month appeared to be more favourable than another. A much reduced amount of flowering occurred during the time of flood water.

(4) *Vossia cuspidata* (Roxb.) Griff. reached its maximum flowering towards the end of November, while its vegetative growth was continuous throughout the year. Being a plant with its principal vegetative growth on the surface of the water it is presumably not much affected by cloudy water.

(5) *Ottelia alismoides* Pers. showed maximum fruiting during June.

(6) *Juncellus alopecuroides* (Rottb.) C.B.Cl. appeared to have two peaks of flowering, viz. mid-May and the end of December, with lesser flowering in the intervening months. It was noticed that this plant did not thrive when planted in the centre of the experimental canals, and subsequent growth from seedlings always occurred in the shallowest water, emphasizing its preference for the habitat noted in the Gezira survey.

Table 1 shows the extent of the plants after 14 months' undisturbed growth. It will be noted that this period has included one flood period, but sufficient time has elapsed since its departure for its immediate effects to have been obliterated.

*Najas pectinata* (Parl.) Magnus had shown the greatest spread, having extended over 111 sections out of a possible 120. This is in conformity with its behaviour in the Gezira. This plant is, perhaps, the most rapid invader of any present in the minor canals and is one of the most effective stoppers of water flow. There is a suggestion that when present in dense masses in a canal it will suppress other aquatic plants.

*Potamogeton perfoliatus* Linn. had spread over ninety-seven sections out of 120 and rivals *Najas pectinata* in its spreading potentialities. It seeds in varying quantity throughout the year, and doubtless in the Gezira canals where there is some flow of water its spread is even more extensive than in these experimental canals. Of the *Potamogeton* spp. present in the Gezira canals this one more than any other prefers shallow, still water and frequently occurs towards the tail of a canal.

*P. nodosus* Poir. has colonized about half the possible area. It appears to be a slower colonizer than the preceding plant, as shown in its distribution in the Gezira, but is more wiry and tenacious when once established. In Egypt its stems are strong enough to impede navigation and the plant can live in fast- or slow-flowing water (Simpson, 1932).

*Vossia cuspidata* (Roxb.) Griff. and *Juncellus alopecuroides* (Rottb.) C.B.Cl. are similar, in capacity for spread, to the latter plant, emphasizing its liking for shallow water by occurring at the tail of canals.

*Ottelia alismoides* (L.) Pers., being an annual, has died out, only one plant being found in the whole system. The plants had by then formed their fruits which disintegrate on the bed of the canal and release the seeds. Seed production is prolific, 3000 having been counted in one large fruit. The plant does not appear to spread rapidly, for after 19 months it has only spread to twenty-three sections from its original planting in fourteen sections (see Table 3). The seed is surrounded with hygroscopic mucilage which would tend to keep it in the place where it was released from the fruit.

TABLE 1. *Number of sections of canals infected after 14 months (canals examined 6–8 Mar. 1939)*

| Canal | *P.P.* | *O.A.* | *J.A.* | *P.N.* | *V.C.* | *N.P.* |
|---|---|---|---|---|---|---|
| A | 10 | 1 | 1 | 9 | 3 | 7 |
| B | 9 | — | 2+T | 5 | 3 | 10 |
| C | 10 | — | 5+T | 3 | 3 | 10 |
| D | 5 | — | 5 | 8 | 5 | 10 |
| E | 10 | — | 7 | 3 | 3 | 10 |
| F | 9 | — | 4 | 9 | 4 | 9 |
| G | 6 | — | 3 | 6 | 3 | 7 |
| H | 8 | — | 3 | 3 | 3 | 10 |
| I | 10 | — | 2+T | 5 | 6 | 10 |
| J | 4 | — | 3 | 7 | 2 | 10 |
| K | 9 | — | 6 | 5 | 5 | 8 |
| L | 7 | — | 7+T | 4 | 6 | 10 |
| Total | 97 | 1 | 48+4T | 67 | 46 | 111 |
| No. of sections originally sown | 12 | 14 | 12 | 12 | 14 | 12 |

T = tail of canal; *P.P.* = *Potamogeton perfoliatus*; *O.A.* = *Ottelia alismoides*; *J.A.* = *Juncellus alopecuroides*; *P.N.* = *Potamogeton nodosus*; *V.C.* = *Vossia cuspidata*; *N.P.* = *Najas pectinata*.

Total number of sections = 120.

At no time were we able successfully to establish *Chara globularis* Thuillier. During the clear-water period it grows in the Gezira canals with remarkable speed. It lines the canal bed and can be so dense that it raises the level of the water, such that water height readings taken at the regulators become fictitious and bear little relation to the quantity of water in the canal. This plant, contrary to many aquatic plants, will sink in water if placed on the water surface. This is possibly associated with the abundant calcium carbonate in its tissues. *Chara* does not appear to survive transplantation on a large scale. It cannot as a rule in the vegetative condition survive the flood-water period. Great care had to be taken when cultivating this plant in the laboratory that the water contained no silt in suspension, otherwise rapid death of the plant ensued.

At the end of the year's observations the following experiments were carried out in the experimental canals during the course of 3 years.

### Exp. I. *Effect of drying and removing surface soil from canal beds*

The canals were drained towards the end of March (when the irrigation of the cotton crop was drawing to a close) and left dry until the flood water arrived on 23 July. Before its arrival, viz. during 8–11 July, the surface soil to different depths was removed from some of the canal beds, in the hope that seed lying on the surface and rhizomes would be removed with it.

TABLE 2

| Depth (in.) | Canals | Control |
|---|---|---|
| 0– 6 | 8·9 | 5·6 |
| 6–12 | 16·0 | 7·1 |
| 12–18 | 16·5 | 12·8 |
| 18–24 | 17·4 | 15·3 |
| 24–30 | 18·3 | 17·5 |
| 30–36 | 18·6 | 18·3 |

The canals were thus without water for a period of $3\frac{1}{2}$ months. Immediately before removal of the surface soil, moisture contents were determined on the soil of the canal beds and of the surrounding dry land as control. Table 2 shows the percentage moisture. The sampling showed that the bed of the canals consisted of an upper layer of 4 in. of silt brought in by the flood water, and lower layers of clay. It will be noted that the layer 6–12 in. still contained over double the moisture of the control even after exposure to the sun for $3\frac{1}{2}$ months.

On 4 July, twenty-eight samples of soil taken at random from the surface of the canal beds were examined for viable seed. It was found that seed of *Potamogeton perfoliatus* Linn., *P. nodosus* Poir., *Juncellus alopecuroides* (Rottb.) C.B.Cl., *Ottelia alismoides* (L.) Pers. and *Najas pectinata* (Parl.) Magnus were able to survive $3\frac{1}{2}$ months' sun drying during the hottest part of the year. Guppy (1894–7) conducted a series of experiments on the effect of drying on viability of *Potamogeton* seeds, and concluded that they retain their power of germination for several months after drying. It would appear that for at least a period of $3\frac{1}{2}$ months even the scorching Sudan sun is unable to kill them. Muenscher (1936*a*) states that seeds with thin pericarps do not withstand drying but usually die, while those with a thick bony pericarp upon drying enter a long dormant period. He found that after 5 months of dry storage at laboratory temperature, 19% germination occurred among seed of *P. nodosus* Poir.

after they had been cut open. Muenscher (1936*b*), on the other hand, tested the seeds of eighteen species of *Potamogeton* (of which *P. nodosus* Poir. was one) that had been air dried for 2 months or longer and found that these gave almost no germination, but that germination was obtained from all species of which undried water-stored seeds were available. Variations in the length of the dormant period may explain the different results obtained.

The surface soil of some of the canal beds was removed on 8–11 July as follows:

| Depth (in.) | Canals |
|---|---|
| 3 | B.H.L. |
| 6 | C.F.K. |
| 9 | A.E.I. |
| Control (no soil removed) | D.G.J. |

The soil was removed in wicker baskets by hand. It was evident during the operation that this was not the most efficient method for ensuring complete removal of seeds and rhizomes.

Subsequent growth of water plants in the canals was at first greatest in the control canals but, later, there was little to choose between them as regards intensity of the plants.

The canals were again drained on 30 Aug. 1939, and it was noticed that all new growth was from seeds with the exception of *Vossia cuspidata* Griff. and *Juncellus alopecuroides* (Rottb.) C.B.Cl. and a few plants of *Potamogeton nodosus* Poir., whose rhizomes were able to survive the dry period (see Table 3).

From this experiment it is evident:

(1) That under the present irrigation system no drying-out period could be introduced which would be sufficiently long to ensure that reinfestation *in situ* will not occur. Drying out of a canal merely causes a temporary delay in its reinfestation.

(2) That removal of the top layers of soil of the canal bed is only a transitory control.

(3) That, though during the flood water period vegetative growth is almost absent, abundant seed germination still occurs (Table 3).

Exp. II (*a*). *Effect of a short flush of water on the germination of seed on the dry canal bed*

The canals were drained and allowed to dry. Some canals were then flooded for 25 days, drained and allowed to dry again until the flood water appeared. The intent of the experiment was to discover if, after the vegetative portion of the plants had been killed by dry exposure to the sun, the seed on the dry canal bed would be germinated by the short flooding period and the seedlings killed by subsequent drying.

TABLE 3. *Number of sections containing seedlings and/or rhizomes* (*canals examined* 30 *Aug.* 1939)

| Depth of soil removed in. | Canal | *P.P.* | | *O.A.* | | *J.A.* | | *P.N.* | | *V.C.* | | *N.P.* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | R | S | R | S | R | S | R | S | R | S | R |
| Nil | D | 7 | — | 4 | — | — | 7 | 8 | — | — | 6 | 9 | — |
| Nil | G | 10 | — | — | — | — | — | 10+T | 2 | — | 3 | 10 | — |
| Nil | J | 10 | — | 10 | — | — | 1 | 8 | — | — | — | 10 | — |
| 3 | B | 10 | — | — | — | — | — | 4 | — | — | 5 | 8 | — |
| 3 | H | 10 | — | — | — | — | 1 | 2 | — | — | 2 | 6 | — |
| 3 | L | 10 | — | 1 | — | — | 7 | 9+T | — | — | 6 | 10 | — |
| 6 | C | 10 | — | — | — | — | — | 3 | — | — | 4 | 5 | — |
| 6 | F | 10 | — | — | — | — | — | 6+T | — | — | 4 | 10 | — |
| 6 | K | 10 | — | 2 | — | — | 2 | 4 | 2 | — | 5 | 3 | — |
| 9 | A | 8 | — | 2 | — | — | — | 7+T | — | — | 1 | 4 | — |
| 9 | E | 10 | — | — | — | — | 9 | 10+T | 2 | — | 3 | 9 | — |
| 9 | I | 10 | — | 4 | — | — | — | 4+T | — | — | 4 | 8 | — |
| Total | | 115 | — | 23 | — | — | 28 | 75+6T | 6 | — | 43 | 92 | — |

S = seed; R = rhizome; T = tail of canal.

*P.P.* = *Potamogeton perfoliatus*; *O.A.* = *Ottelia alismoides*; *J.A.* = *Juncellus alopecuroides*; *P.N.* = *Potamogeton nodosus*; *V.C.* = *Vossia cuspidata*; *N.P.* = *Najas pectinata*.

It was hoped that the seed would be destroyed in this way.

This period of 25 days appeared to be too long, since some of the plants, e.g. *Potamogeton* spp., were able to flower though no seed formation was noticed. Soil samples from the beds of both the dry and flooded canals were taken before the flood water arrived to fill all the canals. Seed-germination tests were made and there seemed to be little difference in the quantity of viable seed between the dry canals and those that had been flooded for a short period and then dried. This was subsequently confirmed by the quantity of the plants that developed after all the canals had been refilled. The effect of the treatment was therefore negligible.

Exp. II (*b*)

This was similar to Exp. II (*a*), but the period of flooding was reduced to 15 days. The result of this experiment was also inconclusive.

Guppy (1894–7) and Muenscher (1936*a*) have noted the delayed germination that occurs among *Potamogeton* seeds. It is possible that owing to this the number of seeds that germinated during the short flooding period in both experiments was small compared with the total seed present. The effect of the treatment would therefore be small and obliterated in the final result.

These two experiments were designed to test control measures that might conceivably be introduced into the present irrigation procedure. It is clear that any control exerted by the experiments on the presence and the growth of the plants was transitory and of little value.

### 5. Laboratory experiments on aquatic plants

Various small-scale experiments were carried out on the effect of dry exposure on the vegetative growth.

Freshly collected specimens were exposed to the sun for 8, 14 and 17 days. After these periods the dry plants were placed in clear water to observe if they revived. After 8 days' exposure vegetative growth of *Najas pectinata* (Parl.) Magnus, *Potamogeton perfoliatus* Linn., *Ottelia alismoides* Pers. and *Potamogeton nodosus* Poir. showed no signs of life.

Seed-germination tests showed as expected that the seeds remained viable: their survival after $3\frac{1}{2}$ months' exposure in the field has already been commented on.

Other experiments were made to determine the effect of poisons on the plants. Fresh plants were mixed together in large dishes having a layer of soil at the bottom. After a few days the clear water was removed from dishes and replaced by the poison solution. After a period of 7 days in the case of the 1/100,000 solutions and 5 days in the case of the 1/10,000 solutions, the plants were washed and placed in fresh water for 8 days to observe if they survived the treatment. The plants tested were *Potamogeton perfoliatus* Linn., *Ottelia alismoides* Pers., *Potamogeton nodosus* Poir. and *Najas pectinata* (Parl.) Magnus. The poisons used in 1/10,000 solution were mercuric chloride, mercuric chloride-iodide, copper sulphate, potassium cyanide, sodium arsenite, bleaching powder, potassium sulphide, sodium silicofluoride and sodium sulphite, with a control solution without poison. In the 1/100,000 solution only mercuric chloride, mercuric chloride-iodide, and sodium arsenite were used.

Under the ideal conditions of stagnant water only mercuric chloride, mercuric chloride-iodide and sodium arsenite in the concentration of 1/10,000 were able to kill the plants, and at 1/100,000 none of the poisons of this experiment was able to kill completely *Potamogeton nodosus* Poir.

Sulphur dioxide gas was pumped into one of the experimental canals, but the quantity required to produce even a slight discoloration of the leaves was so considerable that the use of this gas was not considered an economic proposition. This gas was tested because, should it prove rapidly successful, its presence would not make the water entirely unfit for human and animal consumption.

The work on the use of poisons was discontinued because:

(1) All successful chemicals tested are highly poisonous to man and animals. They could not therefore be introduced in sufficient quantity into the Gezira canals without robbing the inhabitants of the Gezira of their principal water supply.

(2) In order to maintain the required concentration in a flowing system, poison would have to be added continually to the canals. The cost would be immense and poison would be continually washed on to the land with an unknown effect on the growing crops.

(3) It is unlikely that, in the concentrations tested, the successful poisons would kill the seed or deep-seated rhizomes. The killing effect is likely, therefore, to be of only temporary value.

### 6. Land plants invading the canals from the banks

In the foregoing part of this paper attention has been confined to those plants that live in, and cause stoppage of, the water. There are, however, other plants which, while not as a rule interfering with the water flow, tend to encourage during the flood-water period the deposition of silt on the banks and to form stagnant pools where mosquitoes are liable to breed. These plants usually grow on dry land but can live in shallow water, and will invade the canal bed if the water remains sufficiently shallow. The clearance of these plants must therefore be included in the problem under investigation.

The most important invaders in the Gezira are *Cyperus rotundus* Linn. and *Panicum repens* L. The former is essentially a plant of agricultural land, while the latter is always found near perennial water. Both plants grow abundantly on the canal banks and at the water's edge. The former plant spreads by means of rhizomes and abundant small tubers, while the latter has an underground system of rhizomes resembling couch grass in England. Both are very difficult plants to eradicate, particularly when growing in water.

The presence of these plants on the banks above the high-water mark is beneficial in so far as they consolidate the banks.

In the past these plants had been completely weeded from the canal and up the banks to some distance above the high-water mark. This weeding was difficult and costly.

With intent to discover how much of these plants should be removed, an experiment was arranged in Hag Abdulla Subdivision, where the aerial growth of

the plants was removed from the water up to 1 ft. below the high-water mark. It was considered that extending only 1 ft. into the canal water the plants would cause negligible silting. It was necessary, however, to test if mosquito breeding occurred.

By the kindness of Mr D. J. Lewis, Medical Entomologist, Sudan Medical Service, periodic inspections were made of mosquito breeding during the clear-water period. It was not until the inspection carried out during 14–18 Jan. that mosquito larvae were discovered among the plants. Mosquito breeding occurred only where a portion of a canal was opposite land that had been cropped with *Sorghum*. *Sorghum*, the principal grain crop of the Sudan, is a tall grass. The dry leaves and stems after the harvesting of the grain are blown into the canals and caught by the plants at the water edge. These two tend to form stagnant pools where mosquito breeding is possible. A similar condition ensues if these plants are sufficiently infested with *Spirogyra* spp. to form a matted stagnant pool: this condition is comparatively rare. The fact that breeding takes place only opposite *Sorghum* land indicates that the water plants themselves do not permit mosquito breeding. The reason for this, according to Mr Lewis, is that in this narrow 1 ft. strip the larvae are effected by wave action and changes in the water level which expose them to the attacks of predators. The *Sorghum* debris could be removed periodically and mosquito breeding should cease.

Both plants form abundant seed and, while *Panicum repens* Linn. has not been found growing on cotton land, *Cyperus rotundus* L. is one of the major pests of the Gezira cultivated area (Andrews, 1940 *a*, *b*). The seed of the latter may fall into the canal water and be washed on to the cultivated land. In spite of attempts to prove it, no direct evidence has yet been obtained that this does in fact occur, though it seems a most likely possibility. *Cyperus rotundus* L. forms its seed in September and early October. During this period all available labour is employed in weeding the *Sorghum* and cotton crops and none can be spared to clean the canals. This possible menace must of necessity be allowed to take its course.

### 7. Discussion on aquatic plant control

It is clear from the foregoing that no practical means of complete eradication seems possible. Whatever means are adopted for ridding the canals of plants there always remains the likelihood of reinfection from the Reservoir by those plants common to both. It is obvious that the ideal position, both from an irrigation engineer's viewpoint and from that of dealing with the problem on an economic financial basis, would be to control the plants in such a manner that free passage of water was always possible and yet the costs of the clearance were kept at a minimum.

Control and not eradication should be the aim of the clearance method, though constant efforts with the former may in some cases lead eventually to the latter.

The method formerly in use by the Sudan Irrigation Department for the clearance of aquatic weeds was to wait until stoppage of water occurred and then with a large gang of labourers to remove the plants. No further work would then be done on the plants until stoppage recurred when clearance would be carried out again.

From the results of this study of the aquatic plants it is clear that this method is inefficient. The abundant production of seed that occurs annually emphasizes the necessity for dealing with the plants in such a manner as to avoid this potent source of reinfestation. The abundant germination of seed during the flood-water period (see Table 3) when the vegetative parts of the plants are not generally visible emphasizes that the cleaning must also be maintained during the flood-water period, though active measures to prevent seeding may, in time, result in clearance during the flood-water period being unnecessary. It is thus clear that the most promising method of clearance would be a system whereby each infested canal is cleaned at frequent regular intervals by a small gang of men permanently employed throughout the year. By cleaning the canals in rotation, seeding during the clear-water period would be prevented and seedlings formed during the time of flood water would be removed. A rotational system of rather a different type has been developed in Australia for the control of *Typha* spp. (Prunster, 1940).

Observations in the experimental canals indicated that an interval of 10–15 days between each successive cleaning of a canal would be sufficient to prevent seeding, and an interval of 15–20 days to remove seedlings.

This rotational method of control was tested in the canals of Hag Abdulla Subdivision. The method employed and results obtained are described in the following section.

### 8. Aquatic plant control in Hag Abdulla Subdivision

The Hag Abdulla Subdivision lies at the extreme south of the canalization system. It consists (exclusive of the Main Canal) of 245 km. of canals, and while some canals are of recent origin, others are among the oldest canals of the scheme.

The water plants in the Subdivision consisted of *Najas pectinata* (Parl.) Magnus, *Ottelia alismoides* (L.) Pers., *Potamogeton perfoliatus* Linn., *P. nodosus* Poir., *Chara globularis* Thuillier, *Potamogeton pectinatus* Linn., *Vossia cuspidata* (Roxb.) Griff., *Juncellus alopecuroides* (Rottb.) C.B.Cl., *Cyperus rotundus* Linn. and *Panicum repens* Linn. The first two plants were widely scattered over the whole Subdivision, the remainder were more localized. During June 1940 the bulk of the canals were cleaned by the Irrigation Department

in the usual way. From July 1940 onwards the weeding programme in these canals was arranged by us in accordance with the above suggestions.

In Jan. 1942 the remainder of the Subdivision was included in the experiment. On 31 Mar. 1943 the control of the weed clearance with the organization that had been developed was handed back to the Irrigation Department.

It is not proposed to describe in detail the many modifications that were indicated by increasing experience in the clearance of a large total length of canal. The final method employed is given below.

(*a*) *Method of removal of the plants*

Various instruments had been tested in the past by the Irrigation Department for this purpose. They were, however, tested with the idea of dealing rapidly with the causes of water stoppage and not primarily to control the growth of the plants.

It was decided after experiment that a metal rake with a wooden handle would be the instrument used for plant removal in the experimental area. Rakes are cheap and could easily be made locally. After various modifications the following types were finally in use:

(1) A rake with a head $\frac{1}{2}$ m. long with 15 slightly curved prongs each 15 cm. long.

(2) A rake with a head 1 m. long with 7 prongs each 20 cm. long, the ends of the prongs being joined by a knife blade and the holes between the prongs covered by 1 in. chicken wire.

The first type was used for rooting out the rhizomes of *Potamogeton* spp. The instrument was dragged by labourers through the water along the canal bed. The second type was employed for removing vegetative growth and seedlings. The plants were dragged from the canals by labourers standing on the banks, entering the water only in the wider canals. Precautions were taken that the health of the labourers was not impaired by standing in the water.

The plants were cleared in the following manner:

| Water plant | Type of rake used |
|---|---|
| *Potamogeton perfoliatus* Linn. | Type 2 followed by type 1 then type 2 |
| *P. nodosus* Poir. | Type 2 followed by type 1 then type 2 |
| *Chara globularia* Thuillier | Type 2 |
| *Najas pectinata* (Parl.) Magnus | Type 2 |
| *Ottelia alismoides* (L.) Pers. | Hand pulling was best for the larger plants: type 2 was used for seedlings and small plants |
| *Potamogeton pectinatus* Linn. | More efficiently removed by hand |
| *Juncellus alopecuroides* (Rottb.) C.B.Cl. | Plants removed by hand with a Kadunka* |
| *Vossia cuspidata* (Roxb.) Griff. | |
| *Cyperus rotundus* Linn. | |
| *Panicum repens* Linn. | |

* A large type of adze.

(*b*) *The organization for water plant clearance*

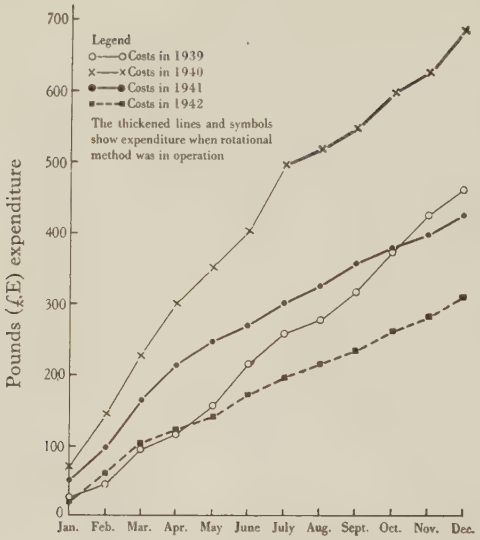
The Hag Abdulla Subdivision was divided into two areas each under a head man. The appropriate division was based on the length of the canal in the area, weed intensity and ease of communication. The head men were controlled by one official who was responsible for the weed clearance in the whole Subdivision. Each head man had under his charge eight labourers as a minimum permanent gang. For successful weed control it was essential that this gang be *permanently* employed on weed clearance, and not diverted to other perhaps more pressing work. The size of this gang was increased as conditions demanded and decreased to the permanent minimum gang as soon as conditions warranted.

Each area was divided into sections and a definite number of labourers allotted to each section, their number depending on the total length of the canal in the section and the degree of infestation. Each set of labourers remained in their particular section and were not transferred elsewhere. They thus became thoroughly acquainted with the heavily infested canals of their section. All the labourers of a section worked together in one canal, and when that was finished proceeded together to the next canal in rotation. The basis of the rotation system consisted of cleaning each infested canal once every 10–15 days during the clear-water period. This would ensure that no seed formation occurred. During the flood-water period an interval of 15–20 days was allowed between each successive cleaning since its purpose was to remove seedlings.

In the case of some water plants, e.g. *Potamogeton* spp., that were not likely to be reintroduced from the Reservoir, the constant organized regular clearance resulted in their complete eradication. This happened in the case of the small area of *P. nodosus* Poir. that occurred in the Subdivision.

The reduction by half of the clearance costs as a result of the introduction of this rotational system is shown in Text-fig. 8.

It will be noted that weed control costs which averaged about £E43 per month in the first half of 1939 rose to about £E81 per month during the first half of 1940. It is obvious that the extent and intensity of weed infestation was increasing rapidly. On 1 July 1940 the rotational system was introduced over most of the Subdivision. In the second half of 1940 which was the period in which experience was being gained in the application of this method, the monthly cost was £E32 against a monthly cost of £E33 for the similar period in 1939. For the first 6 months of 1941 the monthly cost was reduced to about £E48, and for the second half of the year to about £E23 per month. The total expenditure for 1941 was thus less than that for 1939. Finally, in 1942, the monthly cost for the first half of the year




Text-fig. 8. Total weeding costs (Hag Abdulla Subdivision).

was still further reduced to about £E 33 per month, and for the second half to about £E 14.

The constancy of the monthly expenditure in 1942 indicates that the water plants were under control and that water stoppage caused by excessive weed growth was unlikely to occur. It was noticed during the testing of this method that the quantity of weed appearing in the canal diminished under the regular treatment, and it is possible that in succeeding years the costs will be further reduced.

While the final rotational method was in operation the canals were maintained in a condition which always permitted free passage of the water. They were, of course, not completely free of plants but they satisfied the requirements of the irrigation engineer.

This clearance method has now been adopted throughout the canalized area.

I have pleasure in acknowledging the assistance I have received in this work from many officials both British and Sudanese of the Sudan Irrigation Department. I also acknowledge the services of my Senior Technical Assistant, Zein Eff. Abdel Nabi.

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## EXPLANATION OF PLATE 1

Fig. 1. Heavily infested canal with abundant *Vossia cuspidata* (Roxb.) Griff., occasional *Echinochloa stagnina* P. Beauv., and *Juncellus alopecuroides* (Rottb.) C.B.Cl. in foreground.

Fig. 2. An almost pure infestation of *Najas pectinata* (Parl.) Magnus in a small canal alongside the Main Canal.

Fig. 3. A heavy growth of *Cyperus rotundus* Linn. lining the bank of a canal.

Fig. 4. *Panicum repens* Linn. encroaching on the bed of a canal.

Fig. 5. A pure colony of *Potamogeton nodosus* Poir. in Abu Sin Canal, Dolga.

Fig. 6. A portion of the partially submerged island, Sennar Reservoir, showing abundant *Echinochloa stagnina* P. Beauv., some *Vossia cuspidata* (Roxb.) Griff., and very occasional *Polygonum glabrum* Willd.

Fig. 7. Experimental canals. (*a*) Typical unit canal of experiment with *Vossia cuspidata* (Roxb.) Griff. in foreground and *Juncellus alopecuroides* (Rottb.) C.B.Cl. in background. (*b*) *Vossia cuspidata* (Roxb.) Griff. in an experimental canal. (*c*) *Juncellus alopecuroides* (Rottb.) C.B.Cl. in an experimental canal.

(*Received* 29 *February* 1944)

Fig. 1

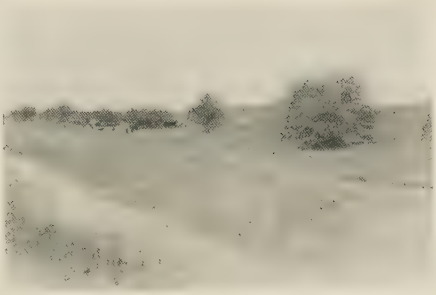


Fig. 2

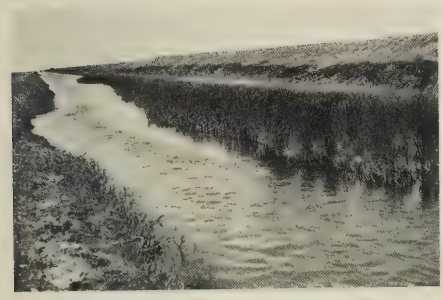


Fig. 3

Fig. 4

Fig. 5

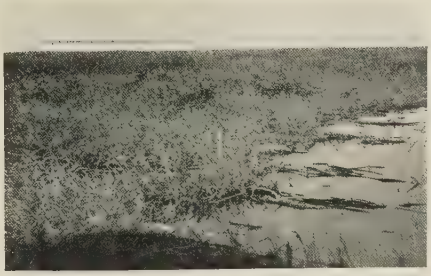
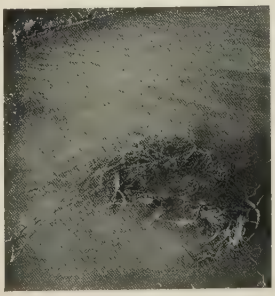


Fig. 6

(*a*)



(*b*)

(*c*)

Fig. 7

# The competition between barley and certain weeds under controlled conditions

By Harold H. Mann and T. W. Barnes, *Woburn Experimental Station, Husborne Crawley, Bletchley*

The nature of the competition between barley and certain annual weeds which are dominant at Woburn, under conditions where the available root space as well as the water, light, and nitrogen required by the plants are limited, has been studied. The weeds involved are *Spergula arvensis* Linn. (spurrey) and *Matricaria inodora* Linn. (scentless mayweed).

Where there is a limited and fixed root space, and where the amount of available nitrogen is also limited, and where barley or each of the two weeds are grown by themselves, there is a tendency for the yield of the crop or of the weeds to reach a maximum with a definite density of plants per volume of soil. When an excess of nitrogenous fertilizer is added, a limit is reached with each of the weeds where extra nitrogen, added as manure, does not increase the growth but only raises the percentage of nitrogen in the plants as grown.

Where there is a constant amount of weediness, with either of the two weeds studied, an increase in the density of barley plants diminishes the injurious effect of the weeds. The combined weight of barley and weeds is hardly ever as great as that of the barley grown in weed-free soil. In a mixed crop, the weight of both weeds steadily decreases as the number of barley plants increases.

Where there is a constant number of barley plants per unit area, the increase of either of the weeds does not much effect the yield of barley till the number of weed plants exceeds the number of barley plants. The presence of a strong, well-grown crop of barley largely reduces the production of spurrey and, to a less extent, that of mayweed. With a constant density of barley and a variable density of weeds, the total weight of barley and weeds, taking the above-ground portion only, is almost constant whatever be the number of weed plants. Where the mixed plants are grown under slight nitrogen stress, the barley secures the greater part of the nitrogen absorbed, obtaining over 75 % of the nitrogen in the combined crop, even when the number of weed plants is greater than the number of barley plants. This applies to both weeds.

If excess of nitrogenous fertilizer (ammonium sulphate) is added to the mixed crop of barley and weeds, above that which will increase the crop, any reduction in the yield of barley *must* be due to competition for root space. At the highest level of nitrogen used (which, however, did not apparently reach the maximum that the barley could utilize for increased growth), the reduction in the yield of barley with excess of spurrey present was only 12 %: with mayweed, a much stronger competitor, it amounted to 45 %.

There is no evidence that with either of the weeds there is any special relationship between barley and the weeds. The effect seems to be only one of competition for root space or for nitrogen when there is not an excess of the latter.

## Introduction

In a study of the weeds prevailing in the area where barley has been continuously grown at the Woburn Experimental Station since 1877, Mann (1939) showed that the two dominant annual weeds on most of the plots were *Spergula arvensis* Linn. (spurrey) and *Matricaria inodora* Linn. (scentless mayweed). The question arose as to how far the decline in yield on the area in question, whatever the manuring, had been due to the inevitable increase in the amount of weeds, especially of those above named. Before directly studying this problem it seemed desirable, in this particular case, to consider whether there is any special relationship between barley and these weeds, or whether the undoubted reduction of crop resulting from their presence was simply the result of competition for the available water, light, nutrients and root space.

During the last 3 years we have conducted experiments with barley and the two weeds named in conditions where the root space was constant but limited; where there was always sufficient water supply, though the soil was well aerated; where the phosphates and potash required by the plants were always in sufficient supply; and where the nitrogenous food supply could be varied. The root space available per plant could, therefore, be varied by altering the number of plants in the given space, and the nitrogenous nutrients were altered at will, but the other supposed factors in the competition remained fixed during the experiments.

## Methods employed

Both barley and weeds were grown in earthenware pots, 28 cm. in diameter, and 25·5 cm. deep, furnished with an upturned outlet near the bottom,

which enabled watering to be done without danger of loss and yet secured good aeration of the soil. The bottom of the pot was covered with coarse gravel to above the outlet. Above this, soil was placed to within 2·5 cm. of the top of the pot, thus giving 16·3 kg. of soil in each case. This gave about 20 cm. depth of actual soil without counting the gravel at the bottom of the pot. Each pot, therefore, contained 12·3 l. of soil, and, if the volume occupied by the gravel be included in that available to the plants, the whole volume would be 14·2 l. in each pot. This space remained constant during the experiments.

The soil was obtained from one of the fields at Woburn, and was a sandy loam, almost free from lime. To this a dose of potassium phosphate, amounting to 0·5 g. $K_2HPO_4$ (1·0 g. in 1943), was added to assure the presence of enough phosphates and potash. The soil was somewhat deficient in available nitrogen but was capable of growing a fair crop of barley without any further addition of nitrogenous manures. However, a dressing of ammonium sulphate was given in all cases, and in one special experiment it was given in various doses.

Barley was sown in the usual way in pot culture, a larger number of seeds being always sown than was required and the excess removed as soon after germination as possible. Though the weeds under study were present as seeds in the soil, it was quickly evident that the best way of using them was to transplant a definite number of seedlings of each of them (obtained from the field) in each pot as required, as soon after germination as they could be handled. All other plants of these or other weeds were removed as soon as they appeared.

The pots were kept well supplied with water throughout the growth of the crop and the weeds, so that, while the soil was always well aerated, the crops and weeds were never under any stress for water. This being the case, the only variables in the experiments were (1) the number of plants per pot, and (2) in one experiment, the amount of nitrogen given as fertilizer. The space available for the roots of the crop or of the weeds and crop together was constant in all cases.

### Competition between different numbers of barley or weed plants

The first question to consider is how far the crop (barley) or the weeds are affected by the number of plants of its own kind per unit volume of soil.

*Barley.* In each of the pots already described, barley plants, varying in number from one to eight, were grown. All the pots were in duplicate. Each pot received 0·5 g. nitrogen in the form of ammonium sulphate 16 days after the sowing of the barley seed on 5 Apr. The barley grew without incident and was harvested on 14 Aug. Table 1 shows the results of the crop.

With the amount of nitrogen available to the plants in this experiment, it is clear that the amount of grain produced per unit of soil space or surface area is the same whether there is one or more plants up to four, i.e. till the root space per plant goes down below 3·5 l. The total produce, similarly, does not decrease in yield till there are more than six plants per pot, i.e. till the root space per plant is less than 2·4 l. With less space per plant than this, the yield is reduced, in spite of the shoots or the number of ears per pot remaining the same or even, in the case of the shoots, going higher.

Table 1. *Results of competition between barley plants grown alone*

| No. of barley plants per pot | Root space per plant l. | Max. no. of shoots | | No. of ears | | Wt. of grain | | Wt. of total produce* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Per pot | Per plant | Per pot | Per plant | Per pot g. | Per plant g. | Per pot g. | Per plant g. |
| 1 | 14·2 | 21 | 21 | 19 | 19 | 29·3 | 29·3 | 65·8 | 65·8 |
| 2 | 7·1 | 31 | 16 | 26 | 13 | 31·3 | 15·6 | 69·5 | 34·7 |
| 3 | 4·7 | 33 | 11 | 26 | 9 | 30·7 | 10·2 | 68·0 | 22·7 |
| 4 | 3·5 | 46 | 12 | 28 | 7 | 31·3 | 7·8 | 70·4 | 17·6 |
| 6 | 2·4 | 51 | 9 | 28 | 5 | 26·5 | 4·4 | 68·3 | 11·4 |
| 8 | 1·8 | 57 | 7 | 30 | 4 | 24·0 | 3·0 | 63·4 | 7·9 |

* In this case the total produce does not include the stubble.

*Spurrey.* An experiment was arranged with spurrey exactly like that just described with barley. The most suitable index of growth while the plants are on the ground is the number of main basal shoots on the spurrey plants. We have thus given this figure as well as the total air-dry matter of the spurrey plants above ground as found on 15 June when the spurrey was going to seed.

It will be seen that spurrey gives an increased crop per unit area or per unit volume of soil till the root space per plant is reduced below 2·4 l., but the amount of nitrogen in the whole crop of weed is remarkably even. This is in the case where there is slight, but not serious, nitrogen stress.

*Mayweed.* This weed seems a little more exacting as to root space than spurrey, as is shown in Table 3.

Here the yield increases with the number of plants per unit area or soil space, but only very slightly.

One plant is able to take as much nitrogen from the amount available in the soil as any larger number, when the root space is limited, as it was here, and where there is slight nitrogen stress. As far as weight is concerned, the eight plants, in spite of the relative shortage of nitrogen, are able to give a somewhat larger yield per unit area than any smaller number.

any rate within the limits of plant density used in our experiments. It became interesting, however, to see how far each of the plants under study would be affected by an increased amount of nitrogenous manure. To test this, in each case, the number of plants per pot was kept within the limit at which the total yield per pot was approximately constant, and

TABLE 2. *Results of competition between spurrey plants grown alone*

| No. of spurrey plants per pot | Root space per plant l. | No. of basal branches | | Wt. of air-dry plants | | Nitrogen in crop per pot mg. |
|---|---|---|---|---|---|---|
| | | Per pot | Per plant | Per pot g. | Per plant g. | |
| 1 | 14·2 | 28 | 28 | 38·5 | 38·5 | 736 |
| 2 | 7·1 | 31 | 16 | 39·1 | 19·6 | 745 |
| 4 | 3·5 | 56 | 14 | 53·2 | 13·3 | 720 |
| 6 | 2·4 | 72 | 12 | 57·3 | 9·6 | 685 |
| 8 | 1·8 | 85 | 11 | 54·9 | 6·9 | 697 |

TABLE 3. *Results of competition between mayweed plants grown alone*

| No. of mayweed plants per pot | Root space per plant l. | No. of basal branches | | Wt. of air-dry plants | | Nitrogen in crop per pot mg. |
|---|---|---|---|---|---|---|
| | | Per pot | Per plant | Per pot g. | Per plant g. | |
| 1 | 14·2 | 11 | 11 | 62·6 | 62·6 | 646 |
| 2 | 7·1 | 19 | 10 | 64·5 | 32·3 | 616 |
| 4 | 3·5 | 34 | 9 | 67·1 | 16·8 | 578 |
| 6 | 2·4 | 44 | 7 | 68·6 | 11·4 | 489 |
| 8 | 1·8 | 59 | 7 | 71·5 | 8·9 | 507 |

TABLE 4. *Results of increasing nitrogen supply on yield of barley or of weeds*

| Crop | No. of plants per pot | Amount nitrogen added per pot g. | Yield and nitrogen content of total produce (excluding roots) | | Nitrogen in total produce, including roots per pot mg. |
|---|---|---|---|---|---|
| | | | Yield per pot (air-dry) g. | Percentage of nitrogen (on oven-dry) % | |
| Barley | 2 | 0·5 | 78·9 | 0·79 | 681 |
| | | 0·75 | 91·6 | 0·85 | 865 |
| | | 1·00 | 99·4 | 1·00 | 1062 |
| | | 1·25 | 109·4 | 1·10 | 1248 |
| Spurrey | 6 | 0·5 | 49·0 | 1·49 | 778 |
| | | 0·75 | 57·2 | 1·58 | 931 |
| | | 1·00 | 61·8 | 1·81 | 1130 |
| | | 1·25 | 61·3 | 2·06 | 1275 |
| Mayweed | 6 | 0·5 | 52·3 | 1·08 | 599 |
| | | 0·75 | 67·7 | 1·08 | 742 |
| | | 1·00 | 70·9 | 1·53 | 1119 |
| | | 1·25 | 71·9 | 1·81 | 1317 |

Thus, in each case, where there is a limited and fixed root space, and where the amount of available nitrogen is also limited, there is a tendency for the yield of the crop or the weeds to reach a maximum with a definite density of plants per volume of soil. If the number of plants is increased beyond this, the yield tends to fall off. This is quite clear with barley and spurrey, but not quite so sure with mayweed, at the amount of nitrogen added, as ammonium sulphate, was varied from 0·5 to 1·25 g. nitrogen per pot. This will show the point, in nitrogen supply, at which each of the plants has as much as the number of individuals grown can use: beyond this point, any further addition of nitrogen will not result in an increase in the yield of the plant grown. Table 4 shows the results for each of the three plants.

When the amount of added nitrogen is greater than from 0·75 to 1·00 g. per pot, almost the whole of the nitrogen, in the case of the weeds, though not so with barley, goes into increasing the percentage of nitrogen in the produce and not to increasing the yield. We may, therefore, conclude that when this amount of nitrogen is added as ammonium sulphate in this experiment, the spurrey or mayweed plants are under little or no nitrogen stress and, consequently, any slackening off of the increase of yield is due to competition for space. In the case of spurrey, the result is specially clear, though with mayweed it is almost equally obvious. We may, in fact, say that for these two plants, the effect of the plants on one grown in each pot, either without any weeds, or with six plants of either of the weeds, in presence of 0·5 g. added nitrogen per pot (as ammonium sulphate), so that there was slight nitrogen stress during the growth of both barley and weeds. We have thus a measure of the effect of six spurrey or mayweed plants on the yield of barley with varying thickness of sowing of the latter. The results with each weed are shown in Table 5.

It is clear that in the case of spurrey, with a limited and constant amount of space for the roots, the effect of increasing the number of barley plants (the number of spurrey plants remaining constant) is to diminish the injurious effect of the weed until, with eight

Table 5. *Effect of varying thickness of sowing of barley on the yield of barley and of weeds*

| No. of plants per pot | Yield of barley per pot: Wt. of grain g. | Yield of barley per pot: Wt. of total produce g. | Yield of weeds per pot. Wt. of air-dry produce g. | Total produce, barley and weeds g. |
|---|---|---|---|---|
| 1 barley : 6 weeds: Barley alone | 29·3 | 65·8 | — | 65·8 |
| Barley and spurrey | 19·1 | 42·0 | 8·4 | 50·4 |
| Barley and mayweed | 11·4 | 25·8 | 20·9 | 46·7 |
| 2 barley : 6 weeds: Barley alone | 31·3 | 69·5 | — | 69·5 |
| Barley and spurrey | 21·9 | 46·8 | 9·5 | 56·3 |
| Barley and mayweed | 16·2 | 40·1 | 16·6 | 56·7 |
| 3 barley : 6 weeds: Barley alone | 30·7 | 68·0 | — | 68·0 |
| Barley and spurrey | 26·1 | 55·4 | 8·0 | 63·4 |
| Barley and mayweed | 16·9 | 45·4 | 14·4 | 59·8 |
| 4 barley : 6 weeds: Barley alone | 31·3 | 70·4 | — | 70·4 |
| Barley and spurrey | 29·9 | 63·5 | 4·3 | 67·8 |
| Barley and mayweed | 17·5 | 45·7 | 14·3 | 60·0 |
| 6 barley : 6 weeds: Barley alone | 26·6 | 68·3 | — | 68·3 |
| Barley and spurrey | 27·9 | 64·7 | 3·2 | 67·9 |
| Barley and mayweed | 19·4 | 53·4 | 10·2 | 63·6 |
| 8 barley : 6 weeds: Barley alone | 24·0 | 63·4 | — | 63·4 |
| Barley and spurrey | 24·7 | 63·7 | 3·2 | 66·9 |
| Barley and mayweed | 16·7 | 50·8 | 9·2 | 60·0 |

another, after the addition of from 0·75 to 1·00 g. nitrogen as ammonium sulphate, is no longer due to competition for nitrogen, but is due to competition for space for the roots.

### Competition between barley and weeds

So far, we have dealt with the amount of barley or weed plants that can be grown on a limited space, without the competition between plants of the same kind leading to a loss of total produce on a specified area or volume of soil. We have now to consider the more important matter of the way in which the advent of weeds on a soil on which a crop of barley is already growing will lower the yield of barley, or, on the other hand, how far a crop of barley will be able to smother the weeds under study.

Let us consider the second point first. A number of barley plants, varying from one to eight, were barley plants, there is no reduction in the yield of barley. The greater the number of barley plants per unit area, the less is the effect of the weed, and the result is very regular. The same effect is noticed with mayweed, though in this case the evil effect of the weed never completely disappears. The whole result is in accordance with the general idea among grain growers that a thick seeding of a cereal crop tends to smother the weed.

A second effect is that the combined weight of the air-dry matter of the barley and the weed, in both cases, is hardly ever as great as that of the barley when the latter is grown alone, but it approximates to it as the number of barley plants increases.

A third point is that the weight of both weeds steadily decreases as the number of barley plants increases. The falling off is very striking in both cases, but it is specially marked with spurrey.

In the above experiment, we had a constant amount of weediness and a variable amount of barley per pot. In that now to be detailed, we have a constant number of barley plants (with similar conditions as regards nitrogen stress as in the previous case) and a variable amount of either of the weeds under study, and we have to see how far the extra amount of weediness affects a barley crop with a fixed number of plants. The number of weed plants varied from one to eight, while the barley, in those pots where barley was grown, remained at four plants per pot. By a defect in the design of the experiment, we are not able to compare the barley grown alone with that in the presence of the weeds, but we can see the effect of the presence of the barley on the weeds themselves. The results are shown in Table 6.

TABLE 6. *Effects of varying thickness of weed growth on the yield of barley and of weeds*

| | Yield of barley per pot | | Yield of weeds per pot. | Total produce |
|---|---|---|---|---|
| No. of plants per pot | Wt. of grain g. | Wt. of total produce g. | Wt. of air-dry produce g. | barley and weeds g. |
| 1 spurrey alone | — | — | 38·5 | 38·5 |
| 1 spurrey and 4 barley | 44·2 | 92·0 | 2·0 | 94·0 |
| 1 mayweed alone | — | — | 62·6 | 62·6 |
| 1 mayweed and 4 barley | 44·2 | 95·8 | 5·8 | 101·6 |
| 2 spurrey alone | — | — | 39·1 | 39·1 |
| 2 spurrey and 4 barley | 41·1 | 89·5 | 4·5 (?) | 94·0 |
| 2 mayweed alone | — | — | 64·5 | 64·5 |
| 2 mayweed and 4 barley | 45·9 | 95·4 | 4·2 | 99·6 |
| 4 spurrey alone | — | — | 53·2 | 53·2 |
| 4 spurrey and 4 barley | 43·1 | 90·3 | 5·6 | 95·9 |
| 4 mayweed alone | — | — | 67·1 | 67·1 |
| 4 mayweed and 4 barley | 44·4 | 95·8 | 7·1 | 102·9 |
| 6 spurrey alone | — | — | 57·3 | 57·3 |
| 6 spurrey and 4 barley | 42·3 | 91·0 | 6·7 | 97·7 |
| 6 mayweed alone | — | — | 68·6 | 68·6 |
| 6 mayweed and 4 barley | 38·3 | 82·6 | 17·8 | 100·4 |
| 8 spurrey alone | — | — | 54·9 | 54·9 |
| 8 spurrey and 4 barley | 38·8 | 85·0 | 10·6 | 95·6 |
| 8 mayweed alone | — | — | 71·5 | 71·5 |
| 8 mayweed and 4 barley | 37·8 | 79·5 | 19·2 | 98·7 |

From the results of this experiment, the following conclusions can be drawn:

(1) Increase of either of these weeds, with a constant and sufficient planting of barley, does not much affect the yield of barley till the number of weed plants exceeds the number of barley plants. This applies to both of the weeds under consideration. When the number of weed plants exceeds that of barley, mayweed is a more serious competitor with barley than is spurrey.

(2) The presence of a strong crop of barley very largely reduces the production of both spurrey and mayweed, especially of the former.

(3) The total production of air-dry matter in the barley and the weed combined, taking the above-ground portions only, is almost constant in both cases, whatever be the number of weed plants.

(4) When there is competition for a limited amount of available nitrogen, the barley takes by far the larger proportion, though this proportion becomes less as the amount of weed increases. In the case of spurrey, where there are four plants of barley to one plant of spurrey, the above-ground proportion of the barley takes 96 % of the nitrogen in the combined growth: where there are four plants of barley to eight plants of spurrey, the barley still obtains 78 % of the nitrogen in the total growth. With mayweed the proportions are respectively 93 and 76 % in the two cases.

### EFFECT OF EXCESS OF MANURIAL NITROGEN ON COMPETITION BETWEEN BARLEY AND WEEDS

In the experiments just detailed, the amount of nitrogen added to the soil in the form of ammonium sulphate was kept constant, and was, judged by the experiments described on pp. 16 and 17, somewhat less than the barley crop could use profitably. This being the case, it has been shown that when this crop and either of the weeds are grown together, the former is a very efficient competitor for the limited amount of nitrogen available, provided a thick planting of barley is present. The question now arises as to how far the barley and the weeds would compete with one another if the amount of nitrogen present was amply sufficient for both of them, i.e. if a portion of the available nitrogen merely went to increase the

nitrogen content of the crops of barley or weeds and did not increase the amount of material produced. If this stage is reached, it is clear that any effect of the association of the crop and the weeds is due to competition for root space and not to competition for nitrogen. An experiment was, therefore, undertaken in which we were able to compare the yield of barley, of spurrey, or of mayweed with the yield when the plants in similar amount are grown together, with varying amounts of added nitrogen, some of which reached approximately the amounts known to produce the maximum growth.

Pots were, therefore, set up in which, with adequate supplies of phosphates and potash, the nitrogen varied from 0·5 to 1·25 g. per pot added as ammonium sulphate, and which were then sown with (1) two plants of barley, (2) six plants of spurrey, (3) six plants of mayweed, (4) two plants of barley and six plants of spurrey and (5) two plants of barley and six plants of mayweed. These grew normally and were carried to harvest. The results in yield are shown in Table 7.

Table 7. *Effects on increasing manurial nitrogen on competition between barley and weeds*

| No. of plants per pot | Amount nitrogen added per pot g. | Yield of barley | | Yield of weed. Air-dry produce g. | Nitrogen in crop and roots | | | |
|---|---|---|---|---|---|---|---|---|
| | | Grain g. | Total produce g. | | Barley mg. | Weeds mg. | Roots mg. | Total mg. |
| 2 barley | 0·5 | 32·4 | 78·9 | — | 551 | — | 130 | 681 |
| | 0·75 | 38·8 | 91·7 | — | 693 | — | 172 | 865 |
| | 1·00 | 41·6 | 99·5 | — | 884 | — | 178 | 1062 |
| | 1·25 | 47·3 | 109·4 | — | 1064 | — | 184 | 1248 |
| 6 spurrey | 0·5 | — | — | 49·0 | — | 678 | 100 | 778 |
| | 0·75 | — | — | 57·2 | — | 838 | 93 | 931 |
| | 1·00 | — | — | 61·8 | — | 1042 | 88 | 1130 |
| | 1·25 | — | — | 61·3 | — | 1174 | 101 | 1275 |
| 6 mayweed | 0·5 | — | — | 52·3 | — | 509 | 93 | 602 |
| | 0·75 | — | — | 67·7 | — | 652 | 90 | 742 |
| | 1·00 | — | — | 70·9 | — | 975 | 144 | 1119 |
| | 1·25 | — | — | 71·9 | — | 1167 | 150 | 1317 |
| 2 barley and 6 spurrey | 0·5 | 27·4 | 62·6 | 17·6 | 349 | 187 | 144 | 680 |
| | 0·75 | 32·7 | 74·8 | 21·3 | 481 | 275 | 184 | 940 |
| | 1·00 | 35·9 | 83·7 | 22·4 | 639 | 328 | 219 | 1186 |
| | 1·25 | 41·8 | 96·4 | 16·1 | 815 | 290 | 172 | 1277 |
| 2 barley and 6 mayweed | 0·5 | 12·2 | 28·9 | 44·0 | 162 | 380 | 88 | 630 |
| | 0·75 | 19·7 | 44·3 | 39·0 | 274 | 431 | 154 | 859 |
| | 1·00 | 22·1 | 47·8 | 37·9 | 332 | 544 | 160 | 1036 |
| | 1·25 | 28·9 | 60·2 | 36·3 | 491 | 568 | 189 | 1248 |

The first thing that strikes one in the above results is the fact that from a constant amount of added nitrogenous fertilizer in a constant volume of soil, the recovery of nitrogen in the plants, whether of barley, spurrey, mayweed, or a mixture of barley and either of the weeds, is almost identical, provided the available nitrogen is in excess of the needs of the growing plants. At the lower levels of nitrogen, the spurrey seems the most efficient utilizer of nitrogen and the mayweed the least. But at the higher levels there is very little difference.

As far as the weeds are concerned, the increase in the quantity of nitrogen above 1·00 g. per pot, or, in most cases, beyond 0·75 g. per pot, leads to no increase in the yield of produce. This applies whether the weeds are grown alone or in admixture with barley. This does not apply to the same extent with the barley, and the yield of this, whether grown alone or in admixture, goes up at least till 1·25 g. nitrogen per pot has been added. It is true, however, in all cases, that a very large part of the additional nitrogen taken up from the higher amounts of manure is devoted to making the plant material richer in nitrogen.

But at least at the two highest levels of nitrogen, the weeds have not increased in amount, and so have not apparently competed with the barley for the available nitrogen, and any diminution in the yield of barley would seem to be due to a competition for root space. It is also clear that, within the limits of the experiment, the higher the amount of available nitrogen present, the better is the barley able to compete for it. In other words, high nitrogen manuring will tend to improve the competitive power of barley in weedy land.

Within the limits of space already described, the percentage reduction in the yield of two plants of barley (total produce) due to the presence of six

spurrey or six mayweed plants is shown in the following statement, for each of the four levels of nitrogen:

| Amount added nitrogen per pot | Percentage reduction in yield of barley (2 plants) due to the presence of 6 weed plants | |
|---|---|---|
| | Spurrey | Mayweed |
| g. | % | % |
| 0·5 | 21 | 63 |
| 0·75 | 18 | 52 |
| 1·00 | 16 | 52 |
| 1·25 | 12 | 45 |

Thus, the higher the amount of available nitrogen, the less is the competition for space of the weed with the barley. The mayweed is a much stronger competitor for the space available than is the spurrey. While six plants of mayweed have been able to reduce the yield of two plants of barley by 45% when nitrogen is available for both, six plants of spurrey have only been able to reduce it by 12% under similar conditions.

### Relationship of the roots of weeds and barley

It has frequently been suggested that with many weeds there is a specific effect of their roots on those of adjoining plants, the roots being either so attracted that the intermixed fibres could hardly be separated, or else they repelled one another. This point was emphasized by Kaserer (1911) from a study of mixed cultures of staple crops. Almost everyone who has studied mixed cultures has noted many of the points emphasized by Kaserer, and any such attractive or repelling effect would have to be superadded to the result of pure competition when plants are grown together in the same soil. The question, in fact, arises for every weed as to whether its effect is simply the result of competition for nitrogen, for space, for light, or for anything else, or whether it is at least partially the result of some special relationship between the roots of the two associated plants. As the two weeds considered in this paper are the most common and dominant weeds in the area at Woburn permanently under wheat or barley, the question was of special interest. Their dominance at Woburn was described by Mann (1939).

It may at once be said that we have not been able to find any trace of any special relationship between the roots of the weeds we have considered in this paper and barley. They both compete with barley for the space at all depths of the pots in which they are grown and some of the roots of both weeds are found in the gravel at the bottom of the pots, i.e. at 25·5 cm. below the surface of the soil. But the proportion of the mass of roots which consists of the weeds gets less as one descends in the pots. Where there were three barley plants and six weed plants in a pot, the proportion of the root mass which consisted of the weed, at various depths, was as follows:

| Depth (in.) | Barley and spurrey | Barley and mayweed |
|---|---|---|
| 4 | 23·8% spurrey | 28·8% mayweed |
| 8 | 16·0% ,, | 31·2% ,, |
| Bottom of pot, 10 | 7·5% ,, | 11·8% ,, |

Too much stress should not be laid on these exact figures, owing to the difficulty of identifying the fibres of the roots of each plant, but they give an idea of the depth at which the greatest development of the roots of each plant is found. There is no doubt that barley and mayweed are much stronger rooting plants than spurrey.

When grown alone, both weeds have a fairly similar root system. Below ground, there is first a tap root which is quite thick just below the surface, much thicker with mayweed than with spurrey. At about an inch below the surface, the root breaks up into a number of fairly stout branches which proceed downward in a slanting direction, and, though they taper distinctly and fairly rapidly, the original branches go down to the extreme depth of the root system. These give off thin branches, and there is little sign of what may be called 'bifurcation' of the roots. The thin branches give off secondary branches which tend to make up the fibrous mat characteristic of the weeds. While, however, the thicker roots of mayweed are of a dark colour and so can easily be identified in the mixed mass of barley and weed roots, the spurrey roots are much more difficult to detect in the root mass. The finer roots of the mayweed have not the dark colour.

When barley and each weed are grown together, both spurrey and mayweed surface roots interlace with barley roots in a criss-cross pattern of threads seen on first exposing the uppermost parts of the root system. Each barley plant sends out root branches in all directions, often across the whole width of the pot. The mayweed and spurrey similarly send out their surface root branches in all directions, and they are seen to pass freely through the network of barley roots as if the latter were not there. With both weeds, fairly gentle pulling serves to draw away the stronger part of the root system from the barley, but, owing to the very brittle character of the finer roots, it is difficult to separate them entirely. But the impression was obtained that there was no specific attraction of the barley roots for the weeds or vice versa.

This was confirmed by examination of the relationship of the finer roots under the microscope. All attempts at differential staining of the roots, using iodine, fuchsin, ferric chloride and haematoxylin, failed to distinguish between the barley and the two weeds, but by treatment of the mixed roots with

caustic soda, a differentiation could be obtained between barley and the weeds. The method finally adopted was to warm the mass of roots with 0·75 % caustic soda for 2 hr. on the sandbath well below boiling-point, slightly acidifying with dilute hydrochloric acid, and then examining under the microscope. This gives a sufficiently transparent root for the structure to be recognized and enables the proportion of each kind of root in a mixed mass to be determined.

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# A comparative study of onion varieties in relation to bolting and yield when grown from sets

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(With Plate 2 and 5 Text-figures)

Two trials are described, each of twenty-five varieties tested during two seasons and the intervening winter storage period. Sets were produced from seed in the first season and planted to produce mature bulbs in the second. In the second seasons of both trials, the effects of time of planting of sets were also investigated as part of a factorial experiment. In the later trial the effects of set size were also tested. Data are presented of varietal differences in bolting, yield, earliness and of behaviour of the sets in storage. The effects of set size and of late planting on these characteristics are also recorded. Some practical recommendations are made: thus, among those tested, varieties were found which bolted little and gave high yields when grown from sets, but it is emphasized that names of varieties as listed in seedmen's catalogues may not be reliable. Efforts are being made to maintain and improve by selection the most satisfactory of these strains. Late planting of sets is not recommended, for, although bolting was effectively controlled by this means, the yields were much reduced. Plants grown from large sets tended to bolt more than those from small, as has been shown by earlier workers; while on the contrary the yields from large and small sets were on the average alike. Comparing different varieties, the highest gross yields of all were produced from the large sets of non-bolting varieties; but for highest yields of bulbs *free from flower stalks* small sets should be used and this is advisable for all varieties. During storage the large sets lost less percentage weight than the small but they sprouted much more, and this is considered the more serious defect. The storage data demonstrate an additional disadvantage of late planting, for this involves longer storage and both sprouting and weight losses increase rapidly during late spring.

## INTRODUCTION

Raising onion plants from sets instead of from seed sown in the open has certain advantages, principally that it enables onions to be grown successfully on land of lower tilth and that attack by onion fly is less severe. Lloyd (1914) summarized the relative advantages of the use of seed and sets in America and stated that, although the cost of planting sets was very heavy, the balance of value of crop to cost of production lay slightly in their favour. In this country the use of sets on a large scale is almost unknown, but among gardeners and allotment-holders their use has in the past been considerable. The supply, however, has practically ceased since the outbreak of war as the sets were almost all imported. It is generally believed that the climate of Britain is unsuitable for set production, and that the low temperature prevalent is responsible for the high percentage of bolting in the plants raised from them. Bolting is, indeed, the chief defect in onions grown from sets. It may be controlled (Thompson & Smith, 1938; Heath, 1943 *a*, *b*) by high- or low-temperature treatment of the sets during storage, but these treatments demand considerable care and involve expense.

The experiments to be described were therefore undertaken to ascertain whether intrinsically 'non-bolting' varieties could be found which could thus be produced in the open in this country without the need for any special treatment. The possibility of reducing bolting by late planting was also investigated.

TABLE 1. *Varieties used in the experiments*

| V | Name | 1940–1 | 1941–2 | Source |
|---|---|---|---|---|
| 1 | A1 | * | * | B |
| 2 | Ailsa Craig | * | * | A† |
| 3 | Bedfordshire Champion | * | * | A |
| 4 | Bedfordshire Champion | . | * | B |
| 5 | Bedfordshire Champion | . | * | C |
| 6 | Blood Red | * | * | A† |
| 7 | Brown Globe | * | * | A† |
| 8 | Brown Globe | . | * | B |
| 9 | Brown Spanish | * | * | A† |
| 10 | Coconut | * | . | F |
| 11 | Danvers Flat | . | * | C |
| 12 | Danvers Globe | . | * | C |
| 13 | Danvers Yellow Flat | * | * | A† |
| 14 | Danvers Yellow Globe | . | * | A† |
| 15 | Deptford | * | . | A |
| 16 | Giant Rocca | * | . | A |
| 17 | Giant Rocca Golden | * | . | A |
| 18 | Giant White Italian | * | . | A |
| 19 | Giant Zittau | * | . | A |
| 20 | Improved Reading | * | . | B |
| 21 | James Keeping | . | * | B |
| 22 | James Keeping | . | * | C |
| 23 | James Long Keeping | * | * | A |
| 24 | Large Tripoli | * | . | B |
| 25 | Prizetaker | * | . | D |
| 26 | Red Italian | * | . | A |
| 27 | Red Wethersfield | . | * | A† |
| 28 | Reliance | * | * | C |
| 29 | Southport Yellow Globe | . | * | A† |
| 30 | Stock 33 | . | * | G‡ |
| 31 | Up-to-Date | * | * | A |
| 32 | White Globe | * | * | A† |
| 33 | White Spanish | * | * | A |
| 34 | White Spanish | . | * | B |
| 35 | White Spanish | * | . | D |
| 36 | White Spanish | * | . | E§ |
| 37 | Yellow Mulhouse | * | . | E§ |
| 38 | Yellow Ebenezer | . | * | A† |

These names are as quoted in the catalogues of the nurserymen from whom they were obtained. Varieties obtained from any one source are similarly lettered in column 3.

† Known to be American seed in 1941–2.
‡ Cambridge School of Agriculture.
§ France.

## DESIGN OF THE EXPERIMENTS

Two trials, each of twenty-five varieties, were carried out during 3 years: 1940–1 and 1941–2. In each trial, sets produced in one year were stored from autumn until spring and then planted to give a crop in the second season. The trials were laid out as three balanced 5 × 5 lattice squares—both during the production of sets from seed and during the crop years. In both trials, the lay-out for the crop year utilized split plots superimposed on the lattice of varieties. In 1941 each main plot was divided into three subplots to which the three planting dates of sets were allotted at random (designated treatments $E_1$, $E_2$ and $E_3$ respectively). In 1942 each main plot was divided into four subplots representing a 2 × 2 factorial experiment (2 planting dates × 2 sizes of sets, i.e. $E_1$ and $E_2$ × Large (L) and Small (S)). The extra pair of treatments, L and S, was introduced in this year to reduce error due to variation in material and also to seek confirmation of the effects of set size found by other workers (Thomson & Smith, 1938; Heath, 1943*a*).

## EXPERIMENTAL

*Production of sets.* The sets used in both trials were produced at Rothamsted Experimental Station on land formerly in use as an allotment. Following autumn digging the land was raked in May immediately before sowing, giving 1–2 in. of very fine tilth with firm soil below. The individual plots measured 9 × 30 in., and paths were left between them to allow access for cultivation. The site of each plot was consolidated by stamping on a board laid on the soil; nine shallow drills 1 in. apart were then scratched on each plot by means of a toothed hoe. The seed was sown by hand, 3–3½ g. per plot (equivalent to about 170 lb. of seed per acre excluding paths), and then covered to a depth of about ¼ in. with fine soil. Finally, the plot was again compressed with the board. By daily watering the surface was kept continuously moist until most of the seedlings had appeared. This method is a departure from normal practice but very even germination resulted, and this practice has been adopted for all subsequent experiments.

The seed was sown on 9 May in 1940 and germination had begun by the 20th. In 1941 germination was practically complete on 24 May, sowing having taken place on the 8th.

A number of the varieties grown in 1940 were no longer available in the following year—among them those obtained from France (V 36 and 37, there used for set production), and also the Italian varieties. The list was therefore reconstructed, the number being made up to 25 with American varieties and duplications, from other seedsmen, of those that had produced the most satisfactory sets in 1940.

Towards the end of August the foliage of some of the varieties had begun to collapse at the neck. The lists of earliness on p. 29 have been constructed on this criterion. The experiments were harvested shortly afterwards (2–4 Sept. in both years) and the sets laid out on the staging of a greenhouse to dry. About a month later (15 Oct. 1940 and 26 Sept. 1941) the dry tops were twisted off and from each variety all sets below 1 g. or above 10 g. weight were discarded together with those which were thick-necked.

In 1940 the remainder were then weighed, counted, and placed in storage. In 1941, they were first graded by weight into equal numbers of 'large' and 'small' sets and from each of these lots, sixty were taken at random and weighed.

The yield of sets of V 29 Southport Yellow Globe, which had germinated very badly, was found to be insufficient to supply the number required for the experiment, and this variety was therefore discarded, its position in the design being taken by a duplicate lot of V 38 Yellow Ebenezer. This is designated V 38′ in the tables.

*Storage of sets.* In both years the sets were stored in trays in a double-walled, darkened shed. The mean temperature of the store between 15 Oct. 1940 and the removal of the first sample for planting ($E_1$) on 22 Mar. 1941 was 11·9° C. For the further 2 months that the $E_2$ and $E_3$ sets remained in store, the temperatures were 10·1 and 12·6° C. respectively. For the equivalent periods in the second experiment (6 Nov. 1941–1 Apr. 1942 for $E_1$, and 1 Apr.–6 May 1942 for the further 5 weeks the $E_2$ sets remained in storage) the temperatures were 8·5 and 11·7° C. respectively. During the very severe weather in the early part of 1942 it was sometimes necessary to heat the store.

*Production of the crop*, 1941. The crop in 1941 was grown at Rothamsted Experimental Station. The site chosen proved to be very unsuitable for a horticultural crop, the land being very heavy and only recently dug up from grass. The mortality in the field was high and the yield of each plant very small; also, towards the end of the season, a number of the plants was destroyed or unearthed by rooks.

On 22 March the sets from each plot were weighed and counted after removal of rotten bulbs. A random sample of twenty was then taken for the first planting ($E_1$) and the rest returned to the store for a further month. The $E_1$ sets were then planted on 25 and 27 March. Each subplot consisted of twenty sets spaced 15 × 4 in. in two rows. The sets were planted so that their tips were just covered.

A second planting ($E_2$) and a third ($E_3$) were made similarly on 24 April and 22 May, respectively.

Inflorescences were first noted on a few plots, all of treatment $E_1$, on 11 June. On 2 August all bulbs then ripe were harvested. The final harvest took place on 5–8 September when all remaining plants were lifted and weighed.

*Production of the crop*, 1942. Because of the poor yields obtained in 1941 more suitable land was sought for this season. By the courtesy of Dr H. H. Mann, this was provided at the Woburn Experimental Station at Husborne Crawley. The soil there is light and a very satisfactory crop was obtained. The method of planting was essentially similar to that used in the previous year. The subplots were somewhat larger, consisting each of twenty-four plants spaced 18 × 4 in. in two rows. The first and second plantings were intended to be made on the same dates as in 1941, but the first was held up until 13–14 April by wet weather and the second made 13 May, so that actually the planting dates lay approximately half-way between those for $E_1$ and $E_2$, and $E_2$ and $E_3$ respectively of 1941.

By 6 August a number of bulbs had ripened completely and these were harvested and weighed, the final harvest taking place 27–29 August. The bulbs were then stacked in a greenhouse in boxes to dry, but it was not possible to weigh them until 2–9 October.

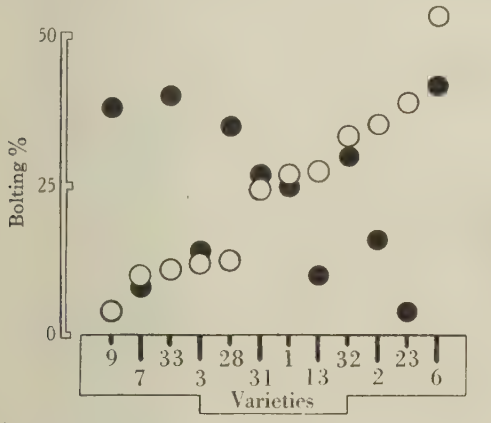
## Results of the trials

The experiments had been designed with the intention of using the method of analysis described by Yates (1940). This proved to give increased efficiency however, only in the analysis of set weights, and for the 1942 data only, in loss in weight of sets in store. The other data have therefore been treated as if derived from three randomized blocks (Yates, 1940). Analyses of percentages based on counts, e.g. of bolting, were carried out using the inverse sine transformation, since the percentages ranged from 0 to 100 and were based on numbers less than 100 (Cochran, 1938). The actual treatment and variety means shown in the tables, however, were obtained from the original data and not by conversion of the means of transformed values back to percentages.

Owing to the very low yields of bulbs obtained in the 1941 trial, these data are unlikely to be of value in assessing the merit of a treatment or variety from a practical standpoint, and the column of yields per unit area is omitted from Table 3.

*Explanation of Tables 2 and 3.* With the exception of those in col. i, Table 3 the figures are, throughout, means over all the subplot treatments. In any column, varieties standing above the *upper blank space* are significantly better than the general mean and those standing below the *lower blank space* significantly worse at the 5 % level of significance. Where no figure is given for 'Significant difference' at the foot of a column, the data have not been analysed. Figures in *italics* are means of transformed values. Column i, 'Yield per acre', is the average yield of the highest yielding subplot treatment (indicated by the symbols in brackets) calculated from the area of the plots exclusive of paths. Column ii, 'Yield per plant', is calculated on the plants surviving to harvest including those harvested early. Column iii, 'Non-bolting yield', is the mean weight of bulbs from plants which did not bolt per twenty-four surviving plants, and gives an indication of the yield of bulbs which do not enclose flower stalks to be expected from twenty-four plants. Column iv, 'Early bolting', refers to the incidence of bolting on 11 June in 1941 and on 4 June in 1942. The percentages are based on the number of surviving plants on those dates. The 'Final bolting' (col. v) is calculated on the

maximum number of plants whose behaviour was known, i.e. no missing plant was left out of the calculations if it had flowered or collapsed at the neck (without flowering) before it was recorded as missing. Column vi, 'Early ripening': in Table 3 the plain figures are the mean percentages of plants which had collapsed at the neck by 16 July, based on the number of survivors on that date which had not bolted. In Table 2 the earliness is gauged by the number of plants which had ripened completely by 1 August (early harvest). The method used in Table 3 probably gives the better estimate, but data of this kind are not available for 1941; for comparison the percentage of plants completely ripe by harvest is given in brackets in Table 3. Column vii, 'Mean set weight', is that at the time the sets entered storage and is the mean of all sets harvested except those discarded because diseased, thick-necked or outside the range



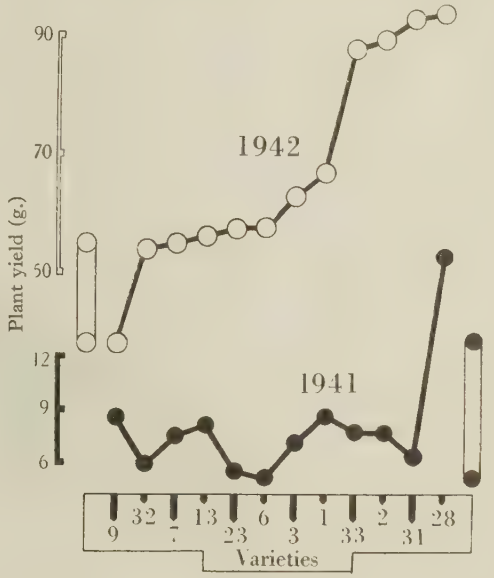

Text-fig. 1. White circles refer to 1942, black to 1941.

1–10 g. Column viii, 'Loss of weight in storage', is the mean percentage loss for sets in store over the period from the beginning of storage until the first samples were removed for planting, and is presented as loss per 4 weeks. Column ix, 'Sprouting in storage', is the mean percentage number of sets sprouted over the period from the beginning of storage up to the time of the removal of the first samples for planting. Column x, 'Mortality in the field', is the percentage of plants which in the crop year failed to survive until harvest, based on the number of sets planted. Such data are not presented for 1942 as mortality then was insignificant even in the worst varieties.

## Discussion and recommendations

*Nomenclature of varieties and strains.* The practical value of these investigations is curtailed by the uncertain identity of the varieties used. The trials have shown that little value can be attached to the name of a strain; two stocks of seed similarly named but procured from different nurserymen behaving very differently as regards yield and bolting. Attention may be drawn in Table 3 to V7 and 8 (both named Brown Globe) in columns ii and v, to V33 and 34 in columns ii, iii and v (both named White Spanish); to V3, 4 and 5 (reputedly Bedfordshire Champion), in columns ii and v; and in Table 2, to White Spanish V33, 35, 36 in column v. All these synonymous varieties show significant differences among themselves. Again, some of these varieties bearing the same name appeared quite distinct also as regards shape and colour.

This confusion is particularly unfortunate because the trials have demonstrated that strains exist which




Text-fig. 2.

bolt so little—even when quite large sets are used—that were guaranteed strains always available the chief difficulty encountered in growing onions from sets would be overcome.

There are also indications here (see Text-figs. 1, 2) that a named variety from the same nurseryman may even behave differently in successive years, and it is possible that the stocks sold are different. The evidence, however, is not very extensive and nearly all the varieties used in both years originated from one source (i.e. 'A'). In the other hand, the differences shown between the bolting behaviours in the 2 years (Text-fig. 1) are especially striking.

In spite of these difficulties it is believed that two varieties, at least, can be recommended with some confidence: V38 Yellow Ebenezer and V28 Reliance. The evidence for this is derived from other experiments in addition to that provided by the trials

TABLE 2. *General varietal characteristics, 1940–1*

| | | ii | | iv | | v | | | vi | | | vii | | viii | ix | | x | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yield/plant g. | | Early bolting % | | Final bolting % | | | Early ripening % | | | Mean set weight g. | | Loss of weight in storage % | Sprouting in storage % | | Mortality in the field % | | |
| V | Key | V | | V | | V | | | V | | | V | | V | V | | V | | |
| | | 37 | 20·4 | 25 | 0 | 26 | 0 | *0·0* | 31 | 93·4 | *81·3* | 23 | 2·03 | 20 1·77 | 20 | 2·9 | *8·1* | 20 | 15 | *19·2* |
| 1 | A 1 | 28 | 17·7 | 26 | 0 | 18 | 1 | *2·3* | 6 | 91·4 | *79·3* | 3 | 2·36 | 37 1·88 | 37 | 4·3 | *11·9* | 28 | 13 | *19·5* |
| 2 | Ailsa Craig | — | — | 17 | 0 | 25 | 2 | *3·0* | 15 | 84·0 | *71·1* | 7 | 2·45 | 36 1·96 | 32 | 5·7 | *13·1* | 19 | 14 | *19·6* |
| 3 | Bedfordshire Champion | 24 | 14·8 | 18 | 0 | 23 | 4 | *5·2* | — | — | — | 13 | 2·53 | 9 2·09 | 19 | 8·1 | *16·4* | — | — | — |
| 6 | Blood Red | 36 | 14·6 | 23 | 0 | 24 | 9 | *11·2* | 32 | 84·3 | *69·8* | 15 | 2·54 | 7 2·14 | 1 | 13·9 | *18·1* | 37 | 22 | *24·6* |
| 7 | Brown Globe | 20 | 12·9 | 16 | ½ | 15 | 8 | *12·3* | 3 | 80·4 | *67·5* | 32 | 2·68 | 3 2·21 | — | — | — | 9 | 19 | *25·4* |
| 9 | Brown Spanish | 19 | 12·9 | 24 | 1 | 17 | 7 | *12·3* | 2 | 79·2 | *66·6* | — | — | 19 2·21 | 7 | 10·3 | *18·8* | 36 | 26 | *28·0* |
| 10 | Coconut | 26 | 12·8 | 15 | 1 | 7 | 8 | *12·4* | 10 | 75·4 | *65·7* | 17 | 2·73 | 35 2·24 | 28 | 10·7 | *18·9* | 13 | 29 | *28·9* |
| 13 | Danvers Yellow Flat | 16 | 11·2 | 7 | 1 | — | — | — | 25 | 75·7 | *65·5* | 18 | 2·79 | — — | 36 | 11·4 | *19·3* | 3 | 27 | *29·2* |
| 15 | Deptford | 10 | 10·4 | 3 | 1 | 13 | 10 | *15·2* | 33 | 75·4 | *65·4* | 36 | 3·06 | 2 2·38 | 23 | 12·8 | *20·0* | 1 | 27 | *30·4* |
| 16 | Giant Rocca | 2 | 8·8 | 37 | 2 | 3 | 14 | *15·8* | 17 | 71·4 | *61·5* | 6 | 3·15 | 28 2·43 | 2 | 14·1 | *21·5* | 10 | 32 | *32·0* |
| 17 | Giant Rocca Golden | 1 | 8·6 | 36 | 3 | 16 | 13 | *16·7* | 35 | 67·8 | *59·0* | 26 | 3·23 | 10 2·51 | 9 | 19·0 | *23·2* | 16 | 38 | *33·0* |
| 18 | Giant White Italian | 9 | 8·6 | 28 | 3 | 2 | 16 | *21·4* | 23 | 71·0 | *59·0* | 1 | 3·29 | 1 2·55 | 10 | 16·6 | *23·7* | 2 | 36 | *34·9* |
| 19 | Giant Zittau | 13 | 8·3 | 13 | 3 | 36 | 17 | *23·7* | 13 | 68·2 | *57·7* | 24 | 3·33 | 23 2·55 | 6 | 19·9 | *23·7* | 33 | 34 | *34·9* |
| 20 | Improved Reading | 17 | 7·6 | 32 | 4 | 1 | 25 | *27·6* | 9 | 61·8 | *55·0* | 35 | 3·33 | 33 2·58 | 33 | 18·4 | *24·8* | 32 | 34 | *35·1* |
| 23 | James Long Keeping | 33 | 7·6 | 1 | 6 | 31 | 27 | *28·9* | 7 | 64·0 | *53·7* | 25 | 3·36 | 31 2·91 | 3 | 17·8 | *25·0* | 7 | 36 | *36·3* |
| 24 | Large Tripoli | 7 | 7·5 | 35 | 7 | 32 | 30 | *29·7* | 1 | 59·6 | *52·1* | 33 | 3·38 | 32 3·17 | 13 | 19·7 | *26·2* | 35 | 38 | *37·0* |
| 25 | Prizetaker | 3 | 7·2 | 2 | 8 | 37 | 30 | *32·5* | 24 | 55·0 | *51·3* | 31 | 3·53 | 6 3·75 | 16 | 23·4 | *28·5* | 31 | 42 | *40·3* |
| 26 | Red Italian | 31 | 6·5 | 31 | 9 | — | — | — | 18 | 56·9 | *50·7* | 9 | 3·74 | 15 4·45 | 35 | 29·5 | *32·8* | 26 | 43 | *40·5* |
| 28 | Reliance | 18 | 6·4 | 10 | 10 | 28 | 35 | *35·8* | 19 | 61·3 | *48·5* | 28 | 3·75 | 17 4·66 | 31 | 29·5 | *33·0* | 18 | 45 | *40·9* |
| 31 | Up-to-Date | 35 | 6·0 | 33 | 11 | 35 | 36 | *36·0* | 16 | 46·4 | *44·3* | 19 | 3·77 | 16 4·67 | 26 | 34·0 | *35·7* | 6 | 46 | *42·1* |
| 32 | White Globe | 32 | 5·9 | 20 | 12 | 9 | 38 | *37·7* | 36 | 35·7 | *40·1* | 2 | 3·77 | — — | — | — | — | 23 | 46 | *42·6* |
| 33 | White Spanish | 25 | 5·7 | 9 | 12 | 6 | 42 | *39·6* | — | — | — | — | — | 13 5·34 | 17 | 38·0 | *37·8* | 24 | 49 | *43·8* |
| 35 | White Spanish | 23 | 5·6 | 19 | 15 | 33 | 40 | *39·7* | 20 | 42·3 | *38·7* | 37 | 3·83 | 24 5·59 | 15 | 47·8 | *43·7* | 15 | 49 | *45·1* |
| 36 | White Spanish | 6 | 5·2 | 6 | 16 | 19 | 44 | *40·9* | 26 | 27·4 | *30·9* | 16 | 4·09 | 18 7·85 | 24 | 50·6 | *45·4* | 17 | 49 | *45·1* |
| 37 | Yellow Mulhouse | 15 | 4·4 | | | 20 | 53 | *46·7* | 28 | 15·8 | *20·2* | 20 | 4·20 | 25 8·38 | 25 | 65·3 | *54·3* | — | — | — |
| | | | | | | 10 | 55 | *48·0* | 37 | 13·8 | *16·3* | 10 | 4·53 | 26 8·46 | 18 | 70·9 | *57·5* | 25 | 72 | *59·4* |
| | Significant difference | | 7·9 | | | | | *12·1* | | | *21·3* | | 0·75 | 1·70 | | | *11·8* | | | *23·2* |

TABLE 3. *General varietal characteristics*, 1941–2

| | | i | | ii | | iii | iv | v | | | vi | | | vii | | viii | ix | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yield/acre tons | | Yield/plant g. | | Non-bolting yield (dekag.) | Early bolting % | Final bolting % | | | Early ripening % | | | Mean set weight g. | | Loss of weight in storage % | Sprouting in storage % | | |
| V | Key | V | | V | | V | V | V | | | V | | | V | | V | V | | |
| 1 | A 1 | 2 | 12·0 ($SE_1$) | 28 | 94·4 | 28 | 202 | 9 | 1 | 9 | 4·0 | *6·4* | 23 | 73·5 | *63·9* (86) | 9 | 2·57 | 34 | 1·50 | 32 | 13·7 | *21·3* |
| 2 | Ailsa Craig | 33 | 11·6 ($LE_1$) | 31 | 92·9 | 33 | 191 | 7 | 1 | 3 | 11·9 | *13·8* | 13 | 70·1 | *60·3* (57) | 3 | 2·72 | 21 | 1·64 | 21 | 16·2 | *23·3* |
| 3 | Bedfordshire Champion | 31 | 11·6 ($LE_1$) | 2 | 89·7 | 38′ | 168 | 33 | 1 | 28 | 12·6 | *14·4* | — | — | — | 33 | 3·18 | 30 | 2·06 | 7 | 18·7 | *25·3* |
| 4 | Bedfordshire Champion | 38 | 11·4 ($LE_1$) | 33 | 88·6 | 31 | 165 | 38′ | 4 | 33 | 10·8 | *14·9* | 4 | 65·0 | *55·4* (83) | 7 | 3·19 | 7 | 2·15 | 30 | 20·6 | *25·9* |
| 5 | Bedfordshire Champion | 28 | 11·4 ($LE_1$) | 38′ | 81·8 | — | — | 38 | 4 | 38 | 13·0 | *15·0* | 30 | 63·4 | *55·0* (79) | 14 | 3·24 | 33 | 2·36 | 34 | 21·1 | *27·1* |
| 6 | Bedfordshire Champion | 38′ | 10·2 ($LE_1$) | 38 | 79·1 | 2 | 142 | 28 | 5 | 7 | 10·4 | *15·6* | 33 | 63·5 | *54·2* (39) | — | — | 38′ | 2·41 | 2 | 21·9 | *27·5* |
| 7 | Blood Red | 5 | 9·4 ($SE_1$) | — | — | 38 | 141 | 3 | 6 | 38′ | 16·9 | *18·2* | 1 | 61·6 | *53·7* (77) | 22 | 3·40 | 38 | 2·43 | 9 | 28·6 | *32·3* |
| 8 | Brown Globe | 8 | 9·1 ($SE_1$) | 11 | 79·0 | 11 | 139 | 32 | 8 | — | — | — | 34 | 59·8 | *51·9* (46) | 38 | 3·53 | 22 | 2·48 | 12 | 30·5 | *32·8* |
| 9 | Brown Globe | 1 | 9·0 ($SE_1$) | 8 | 74·0 | 3 | 133 | 30 | 10 | 31 | 24·4 | *24·3* | 22 | 58·8 | *51·5* (58) | 28 | 3·61 | 13 | 2·50 | 38′ | 30·7 | *32·9* |
| 11 | Brown Spanish | 11 | 8·8 ($SE_1$) | 1 | 66·7 | 1 | 120 | 2 | 11 | 11 | 26·6 | *25·4* | 38 | 59·0 | *50·7* (57) | 13 | 3·62 | — | — | — | — | — |
| 12 | Danvers Flat | 34 | 8·6 ($SE_1$) | 34 | 66·1 | 34 | 109 | 11 | 11 | 1 | 27·0 | *26·6* | 3 | 58·8 | *50·5* (56) | 8 | 3·64 | 28 | 2·53 | 38 | 33·3 | *34·4* |
| 13 | Danvers Globe | 27 | 8·3 ($SE_1$) | 3 | 63·4 | 7 | 109 | 1 | 12 | 13 | 27·5 | *27·4* | 12 | 56·7 | *50·5* (58) | 5 | 3·68 | 1 | 2·56 | 28 | 33·4 | *35·2* |
| 14 | Danvers Yellow Flat | 6 | 8·2 ($SE_1$) | 27 | 63·2 | 21 | 107 | 31 | 13 | 14 | 30·1 | *30·0* | 8 | 48·2 | *47·7* (51) | 27 | 3·71 | 12 | 2·70 | 33 | 34·5 | *35·9* |
| 21 | Danvers Yellow Globe | 3 | 7·8 ($SE_1$) | 30 | 61·3 | 30 | 100 | 14 | 13 | 30 | 32·7 | *31·5* | 11 | 53·2 | *47·0* (45) | 11 | 3·75 | 9 | 2·71 | 31 | 36·1 | *36·8* |
| 22 | James Keeping | 12 | 7·6 ($SE_1$) | 12 | 60·8 | 13 | 100 | 13 | 14 | 32 | 33·5 | *32·1* | 21 | 53·6 | *47·0* (53) | 12 | 3·81 | 11 | 2·71 | 13 | 41·2 | *39·8* |
| 23 | James Keeping | 23 | 7·6 ($SE_1$) | 22 | 60·3 | 5 | 96 | 21 | 15 | 2 | 35·4 | *33·0* | 38′ | 50·3 | *45·3* (49) | 23 | 3·89 | 32 | 2·85 | 22 | 41·4 | *40·0* |
| 27 | James Long Keeping | 14 | 7·4 ($SE_1$) | 21 | 59·1 | 14 | 94 | 34 | 16 | 22 | 36·6 | *33·6* | 28 | 47·5 | *44·0* (33) | 32 | 3·98 | 8 | 2·98 | 3 | 46·2 | *42·8* |
| 28 | Red Wethersfield | 30 | 7·3 ($LE_1$) | 6 | 57·9 | 12 | 92 | 12 | 17 | 21 | 34·9 | *34·7* | 6 | 47·6 | *43·7* (53) | 31 | 3·99 | 27 | 3·06 | — | — | — |
| 30 | Reliance | 13 | 7·3 ($LE_1$) | 23 | 57·5 | 22 | 88 | 5 | 17 | — | — | — | 32 | 39·3 | *38·4* (57) | — | — | 6 | 3·09 | 1 | 52·2 | *46·2* |
| 31 | Stock 33 | 4 | 7·2 ($SE_1$) | 5 | 57·3 | 8 | 85 | 23 | 18 | 12 | 39·3 | *36·6* | 5 | 34·3 | *35·1* (55) | 30 | 4·14 | — | — | 8 | 52·5 | *46·4* |
| 32 | Up-to-Date | 21 | 7·2 ($SE_1$) | 4 | 57·1 | 9 | 85 | 8 | 18 | 23 | 38·9 | *36·9* | 27 | 34·3 | *32·6* (48) | 1 | 4·18 | 5 | 3·20 | 11 | 53·9 | *47·3* |
| 33 | White Globe | 7 | 7·0 ($LE_1$) | 13 | 56·5 | — | — | 22 | 19 | 5 | 38·9 | *37·3* | — | — | — | 6 | 4·18 | 3 | 3·28 | 5 | 58·3 | *50·0* |
| 34 | White Spanish | 22 | 6·7 ($LE_1$) | 14 | 54·9 | 23 | 83 | 27 | 19 | 34 | 39·2 | *37·4* | 14 | 31·1 | *31·7* (58) | 21 | 4·29 | 2 | 3·55 | 27 | 65·8 | *54·5* |
| 38 | White Spanish | 32 | 6·0 ($SE_1$) | 7 | 54·9 | 27 | 82 | 6 | 24 | 4 | 47·3 | *42·5* | 31 | 29·4 | *29·5* (33) | 2 | 4·33 | 14 | 3·58 | 14 | 67·6 | *55·5* |
| 38′ | Yellow Ebenezer | 9 | 5·0 ($LE_1$) | — | — | 6 | 72 | 4 | 24 | 27 | 49·5 | *45·3* | 2 | 25·3 | *26·0* (30) | 4 | 4·41 | 4 | 3·82 | 6 | 68·1 | *55·9* |
| | | | | 32 | 53·7 | 32 | 71 | | | 8 | 50·7 | *46·7* | 9 | 11·0 | *16·1* (42) | 34 | 4·50 | 31 | 3·93 | 23 | 75·6 | *60·9* |
| | | | | 9 | 38·2 | 4 | 68 | | | 6 | 53·5 | *47·5* | 7 | 11·7 | *14·2* (26) | 29 | 4·50 | 23 | 4·26 | 4 | 82·2 | *65·5* |
| | Significant difference | | | | 17·0 | | 42 | | | | | *8·6* | | | *14·0* | | 0·51 | | 0·39 | | | *8·8* |

(actually Ebenezer does not appear at all in the 1941 data), and throughout they have proved reasonably consistent in producing large bulbs, in bolting little and also in shape and colour. For Ebenezer this is true even of seed obtained from another seedsman. In behaviour these varieties are to some extent complementary, for V28 does best in a hot dry season, V38 under cooler conditions. Ebenezer (V38) has been selected in America for set production under conditions of summer day length shorter than those reached in this country. When grown here it therefore behaves as an early type, bulbing beginning early in summer while the days are still short, and ripening as the days lengthen (Heath, 1943*b*, p. 318). The tendency to bulb early is enhanced by high temperatures so that in a hot season premature ripening occurs and yields are reduced. Reliance, on the contrary, is very late, so that in a wet, cool, season difficulty in ripening is experienced, and for the same reasons this variety is unsuitable for northern England and Scotland. In view of the necessity for a period of high temperature in late summer to affect ripening the *production* of sets should be confined to the south and east of England for either variety.

In 1942, when the difficulties with regard to named varieties became apparent, an attempt was made to perpetuate the most promising strains. Seed of White Spanish (V36) and Yellow Mulhouse (V37) remaining from the 1941 trial was also sown, as these varieties were commonly used in France for commercial set production, and since the fall of France have no longer been available. From the 1942 trial V28, 33 and 38 were selected on the basis of their high yield and freedom from bolting. Further selection within the stocks of these was made by saving from the trial only bulbs from such plants of treatment $LE_1$ as had not flowered, this being the treatment most conducive to flowering (p. 32). These bulbs were planted out and seed harvested from them in 1943, but it has yet to be ascertained whether their desirable characteristics are maintained in the progeny.

*Varietal characteristics.* The primary consideration in the selection of onion varieties from the trials must, of course, be yield; and, for practical purposes, yield per unit area of land. This gives an indication of the return to be expected from planting a given number of sets. In Table 3 the first column shows yields calculated on an area basis for the treatment giving the highest yield of each variety; for example, the first entry shows that the highest yield of all was produced by plants of Ailsa Craig grown from small sets planted early. Calculated on an area basis, yield is, however, a composite characteristic depending in part on mortality, so that for theoretical purposes mean yield per plant is of greater interest (column ii, Tables 2 and 3). The order of varieties, in columns i and ii of Table 3 corresponds very closely, however, because the mortality in 1942 was very low, reaching only 9% for the worst variety in this respect (V9). It has been mentioned that sets are mostly used by private gardeners, but where it is intended to market the crop, the appearance of the bulbs as well as their gross yield is important. In Table 2 the third column shows the yield of bulbs from plants which did not flower, since bulbs enclosing the remains of scapes are of poor appearance; also such bulbs tend not to keep (unpublished data).

Considering all the data the following general rule may be stated: varieties with a high incidence of bolting are likely to produce low yields. An outstanding exception to this rule is Ailsa Craig V2, which yielded heavily and occupies the first place in column i in spite of the fact that considerable bolting occurred. Plants which bolt do later form bulbs, but these are relatively small: thus in 1942 bolting plants showed a mean bulb weight of 66·2 g. as against 75·2 g. for those which did not bolt. Ailsa Craig shows similar differences: 79·5 and 105·2 g. respectively and owes its unique position to an inherent high yielding capacity. Otherwise, varieties high up in the yield column also occur near the top of columns iv and v, that is, they show little bolting; as bolting increases yield diminishes. There are, however, some varieties, for example V7 and 9 which produced very low yields although they showed very little bolting. Evidently these are inherently slow growing types, which produce low yields as well as very small sets.

It should be noted that from one point of view the bolting figures are not strictly comparable because the mean set size varied from variety to variety also, and, as has been demonstrated by many workers, large sets tend to flower more than small ones. For practical purposes, however, these are the relevant data because set size is itself a varietal character (as shown in column vii of Tables 2 and 3). For example V9, as already mentioned tended inherently to produce small sets and therefore bolted little. Further, the variation of bolting incidence among the varieties is not solely attributable to the variation in set size; this is demonstrated in Text-fig. 3 where, for each variety, percentage flowering has been plotted against the mean set weight at harvest. The distribution of points clearly shows an upward trend towards the right-hand side of the diagram, but there are obvious varietal differences also; e.g. V28 in 1942 with an average set weight had an exceptionally low bolting incidence, which may be contrasted with that of V8 Brown Globe at approximately the same set size. The characteristic varietal set size tends to affect the subsequent yield in two ways: the yield of varieties producing large sets is apt to be depressed by an increased tendency to flower, but on the other hand, in the absence of bolting larger sets produce larger bulbs.

The set weights recorded in Table 3 for varieties 1, 3, 28, 33 and especially 29 are probably not charac-

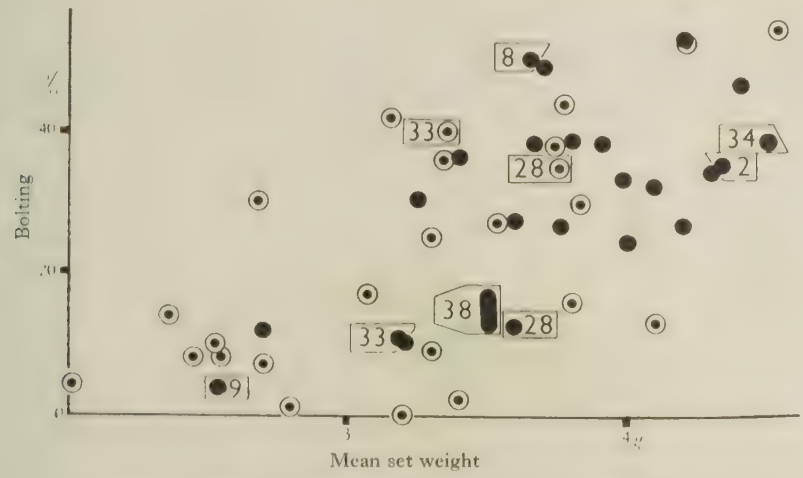
teristic because a large number of sets had been discarded as thick-necked, or the spacing of the seedlings was wide, consequent on a poor stand.

The sets of varieties ripening early were often small, probably for the reasons discussed by Heath (1943*b*, p. 318) in connexion with American strains. Sets of such varieties are easy to produce, but if too small do not withstand adverse conditions in the second season and thus one of the main advantages of using sets is lost. On the other hand, among the late ripening varieties (which tend to give larger sets) a proportion of thick-necked sets is always to be expected, and in a cold season difficulty may be experienced in ripening sets at all. The following classifications are based on the approximate proportion of sets completely ripe by harvest, and should be compared with the order of earliness in the second season shown in column vi of Tables 2 and 3:

| | | |
|---|---|---|
| For 1940–1: | Early | V 3, 7, 13, 15, 23 |
| | Medium | V 1, 6, 9, 17, 18, 24, 25, 28, 31, 32, 33, 35, 36 |
| | Late | V 2, 10, 16, 19, 20, 26, 37 |
| For 1941–2: | Early | V 3, 4, 11, 12, 22, 23, 33, 38 |
| | Medium | V 5, 6, 8, 9, 13, 14, 27, 28, 32 |
| | Late | V 1, 2, 7, 21, 29, 30, 31, 34 |

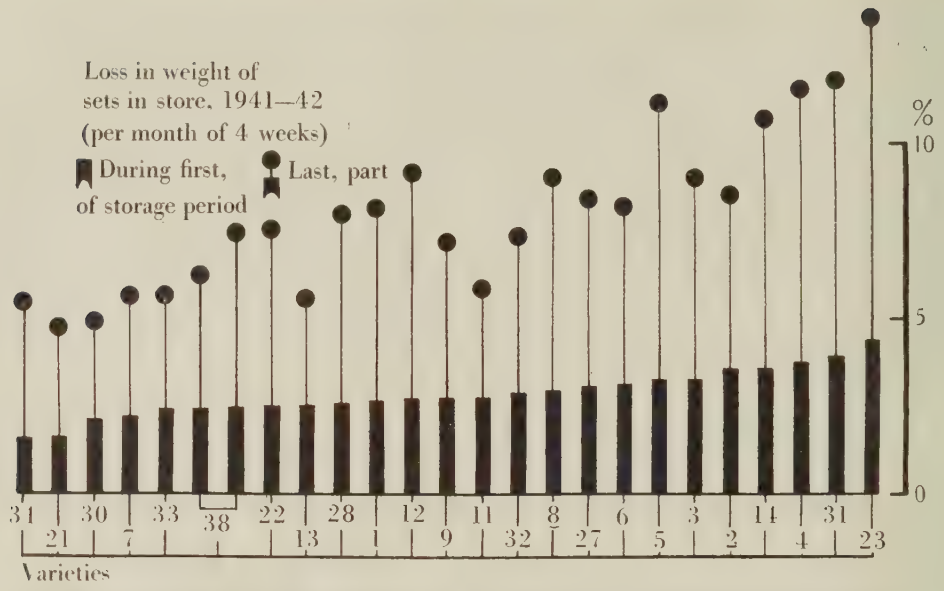
It is often said that American varieties are early and in England tend to ripen prematurely and produce small bulbs. In the present case, however, most of the types used which were known to be of American origin were found to be among the later varieties. Magruder & Allard (1937) in their trials found European types to be later than those used in America. It should, however, be realized that among their European types no English seed was included. In the present trials the order of ripening among the American types used agreed substantially with that found by Magruder & Allard in their work: Danvers Yellow Flat (V 13) and Yellow Ebenezer (V 38), which they found needed only 13 hr. daylight to induce bulbing, are shown in the trials as medium-early. The others, which needed from 13½ to 14½ hr. (the 14½ hr. group included the latest American varieties tested), are shown here to be late or medium late.

*Varietal differences in storage behaviour.* The use of sets entails storage from approximately October to March and varietal differences in keeping qualities are therefore of interest. Figures for loss of weight and for sprouting are presented in Tables 2 and 3. No data for loss by rotting are presented since such losses were very small; however, it is of interest that very many of the highly sprouted sets had to be discarded because of infection with 'neck rot'. This was especially the case during the 1940–1 storage period. The loss of weight data are calculated as the mean over the whole period from the time storage began. Actually the rate of loss rises as the storage period advances and the loss subsequent to the first planting was very rapid indeed (Text-fig. 4). The excessive rate of loss of weight at the end of storage is probably partly caused by the increase in the number of sprouted sets (Text-fig. 5).

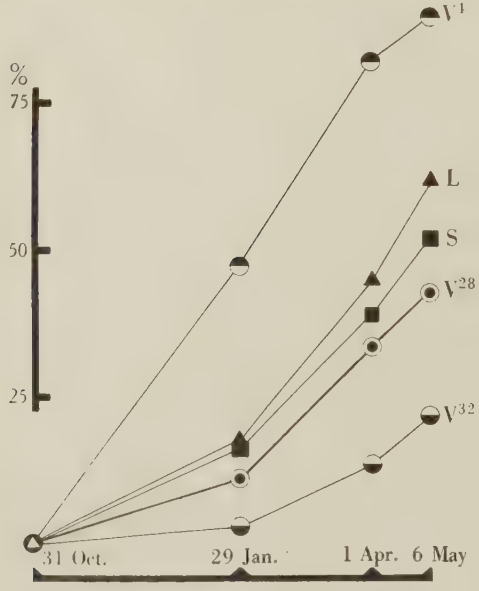



Text-fig. 3. The white circles are from 1941 data, the black from 1942.

*Varietal types.* In the bulletin issued by the Ministry of Agriculture and Fisheries on onion culture (1938), varieties are classified into a number of 'types', but considering the uncertainty of named varieties, it would not have been surprising if no well-defined types were noticed among the varieties tested in the trials. The Italian varieties (V 16, 17, 18, 24, 26) did, however, form a fairly homogeneous




Text-fig. 4. The rates of loss are represented, in each case, by the total heights from the base line.




Text-fig. 5. Sprouting in storage 1942.

group. They were all practically free from bolting, but are unsuitable for use as sets because they stored very badly with a high incidence of sprouting and a correspondingly high loss of weight.

*Time of planting.* From the results of a greenhouse experiment using controlled day lengths Heath (1943*b*) concluded that both long days and high temperatures suppress the emergence of inflorescences, and it was hoped that the trials would show that this could be made use of in practice by late planting. In both years, flowering was substantially reduced by late planting and this may be attributed to the effect of the long summer days combined with relatively high temperatures (cf. Heath & Holdsworth, 1943). In 1942, however, this advantage was completely offset by the very much lower yield obtained from the later planting, whereas in the 1941 trial, the yield rose consistently with the reduction in flowering over the three successive plantings (Table 4).

The lowered $E_2$ yields in 1942 are consistent with the observation (Heath, 1943*b*) that leaf emergence ceases shortly after the onset of bulbing so that, with early bulbing, small bulbs are obtained as a result of the small total leaf area produced. The mean temperature during the growth period of the $E_2$ plants was higher than that of the $E_1$ plants and the day lengths to which the plants were exposed when they first came up, were longer. Thus the onset of bulbing, which is controlled by day-length and temperature (Thomson & Smith, 1938; Heath, 1943*b*) would be expected to take place after a shorter period of growth. The 1941 data are more difficult to explain and do not accord with general experience, but a possible explanation is that the yields of all plants being so extremely low the depression of yield by flowering was a greater proportion of the total yield and so

offset the effect of early planting on the bulb size. For practical purposes only the 1942 data are worthy of consideration and late planting of sets cannot be advised.

The yield curves in Text-fig. 6 show clearly that for the times of planting investigated (mid-April and mid-May) the better yield was always obtained by early planting and this was true even for the varieties showing most bolting. It was also true of all varieties for 'non-bolting yield'. The interaction of variety and planting date effects on yield is, however, just significant and shows (Table 5) that the higher the potential yield of a variety the greater is the loss in yield that may be expected through late planting.

data for 1942. Here the mortality generally was very low, but the losses were actually higher for the late planted sets (a mean of 4% for $E_2$ and 0·8% for $E_1$) this difference probably being due to the excessive sprouting which had taken place by the time the second planting was made. The increase in sprouting towards the end of storage has already been commented on and is a further inducement to plant early. It should be noted, however, that it is not possible to ascribe the contrary effects of late planting on yield in the two years to these contrary effects on mortality, since the data considered were of yield per plant.

*Effects of size.* A list of the mean set weights for

TABLE 4. *Effects of late planting on bolting and yield*

| | | $E_1$ | | $E_2$ | | $E_3$ | | Sig. diff. |
|---|---|---|---|---|---|---|---|---|
| 1941 | Bolting (%) | 34 | *32·0* | 22 | *23·6* | 12 | *15·7* | *3·8* |
| | Yield/plant (g.) | 7·9 | | 9·0 | | 11·6 | | 1·0 |
| 1942 | Bolting (%) | 37·6 | *35·0* | 22·6 | *23·0* | — | | *Not sig.** |
| | Yield/plant (g.) | 92·8 | | 40·6 | | — | | Not sig.* |

*Figures in italics* are transformed values.

* When tested against the appropriate significant higher order interaction as error.

TABLE 5. *Difference between mean yields of* $E_1$ *and* $E_2$

| | | | | | |
|---|---|---|---|---|---|
| V 28* | 73·1 | V 34* | 40·6 | V 23* | 51·6 |
| 31 | 84·7 | 3 | 57·8 | 5 | 48·6 |
| 2 | 72·7 | 27 | 47·0 | 4 | 47·8 |
| 33 | 69·2 | 30 | 52·5 | 13 | 41·0 |
| 38′ | 64·9 | 12 | 39·0 | 14 | 48·2 |
| 38 | 80·4 | 22 | 35·9 | 7 | 37·6 |
| 11 | 55·3 | 21 | 33·9 | 32 | 32·4 |
| 8 | 48·2 | 6 | 33·6 | 9 | 35·0 |
| 1 | 73·7 | | | Sig. diff. | 33·0 |

* Arranged in order of mean yield over all treatments.

It would appear, then, that when a high-yielding variety is being used, early planting is the more to be recommended.

During the 1941 trial there was a heavy mortality of plants in the field, some of the causes of which have been mentioned (p. 24). The early plantings, being subjected to these hazards for a longer period, suffered greater losses. The $E_1$ sets were subjected also to very cold wet weather after planting. Early planting then, would not appear to be advantageous (Table 6).

TABLE 6. *Effects of late planting on survival of plants in the field*

| $E_1$ | $E_2$ | $E_3$ | |
|---|---|---|---|
| 51 | 35 | 20 | Mortality % |
| *45·5* | *35·1* | *23·5* | Sig. diff. *4·4* |

However, during this trial the conditions were much more severe than is ordinarily to be expected and again, therefore, notice must rather be taken of the

TABLE 7. *Interaction of variety and set size on bolting*

| | Bolting % | | | | |
|---|---|---|---|---|---|
| V | L | S | V | L | S |
| 27 | 78·6 | 20·4 | 14 | 53·9 | 6·4 |
| 6 | 78·5 | 28·6 | 13 | 51·4 | 3·5 |
| 8 | 78·3 | 16·2 | 11 | 50·3 | 2·8 |
| 4 | 72·3 | 22·2 | 1 | 49·7 | 4·3 |
| 12 | 67·3 | 11·2 | 31 | 46·1 | 2·8 |
| 5 | 67·2 | 10·6 | 38′ | 33·1 | 0·8 |
| 22 | 66·0 | 7·2 | 38 | 26·0 | 0·0 |
| 34 | 63·5 | 14·9 | 28 | 25·1 | 0·0 |
| 23 | 63·4 | 14·3 | 3 | 23·1 | 0·7 |
| 2 | 62·4 | 8·5 | 33 | 20·1 | 1·4 |
| 30 | 59·7 | 5·6 | 7 | 18·6 | 2·2 |
| 21 | 59·0 | 10·8 | 9 | 8·0 | 0·0 |
| 32 | 56·7 | 10·3 | | | |

the treatments L and S of each variety is shown in Table 9.

It is well established (Thomson & Smith, 1938; Heath, 1943*a*) that, for a particular variety, plants grown from large sets tend to bolt more than those from small sets. In the 1942 trial this was confirmed for all the varieties investigated (Table 7). The individual differences in bolting could not, however, be shown to be significant statistically, as the analysis was on transformed values and further, the average effect of size was not shown significant when tested against the approximate interaction (E × L) as error. No average effect of size on yield (per plant) could be shown; in fact the variance due to size effect was much smaller than the random variance. The reason for this is shown by the values for the highly signi-

ficant interaction between size and time of planting in which small sets gave, on the average, a higher yield than large ones when planted early but a lower one when planted late. This means that for the early planting the bolting was the controlling influence, but that at the late planting bolting was so reduced that the normal tendency of a large set to produce a large plant became apparent. In practice, the superiority of large sets in the later planting is of no consequence as it is always much better to plant early and this is true of all the varieties (Table 8 and also see Text-fig. 6 and Plate 2). Within the early planting, whilst for most varieties the higher yield was given by plants from small sets, for some (V7, 9, 13, 28, 30, 31, 33, 38) it is preferable to use large sets. These are the varieties showing least bolting and include the varieties giving the highest average yields (except, again, V2). For practical purposes then, the rule is: the largest gross yields are obtained by early planting of large sets of varieties giving the highest *average* yields.

TABLE 8. *Interaction effects of set size and planting date on yield*

| | L | S | |
|---|---|---|---|
| $E_1$ | 89 (104) | 96 (203) | Sig. diff. |
| $E_2$ | 45 (64) | 36 (83) | 8 (18) |

Plain figures are yield per plant (g.) In brackets, dekag. of non-flowering bulbs per 24 plants.

TABLE 9. *Mean set weight (g.)*

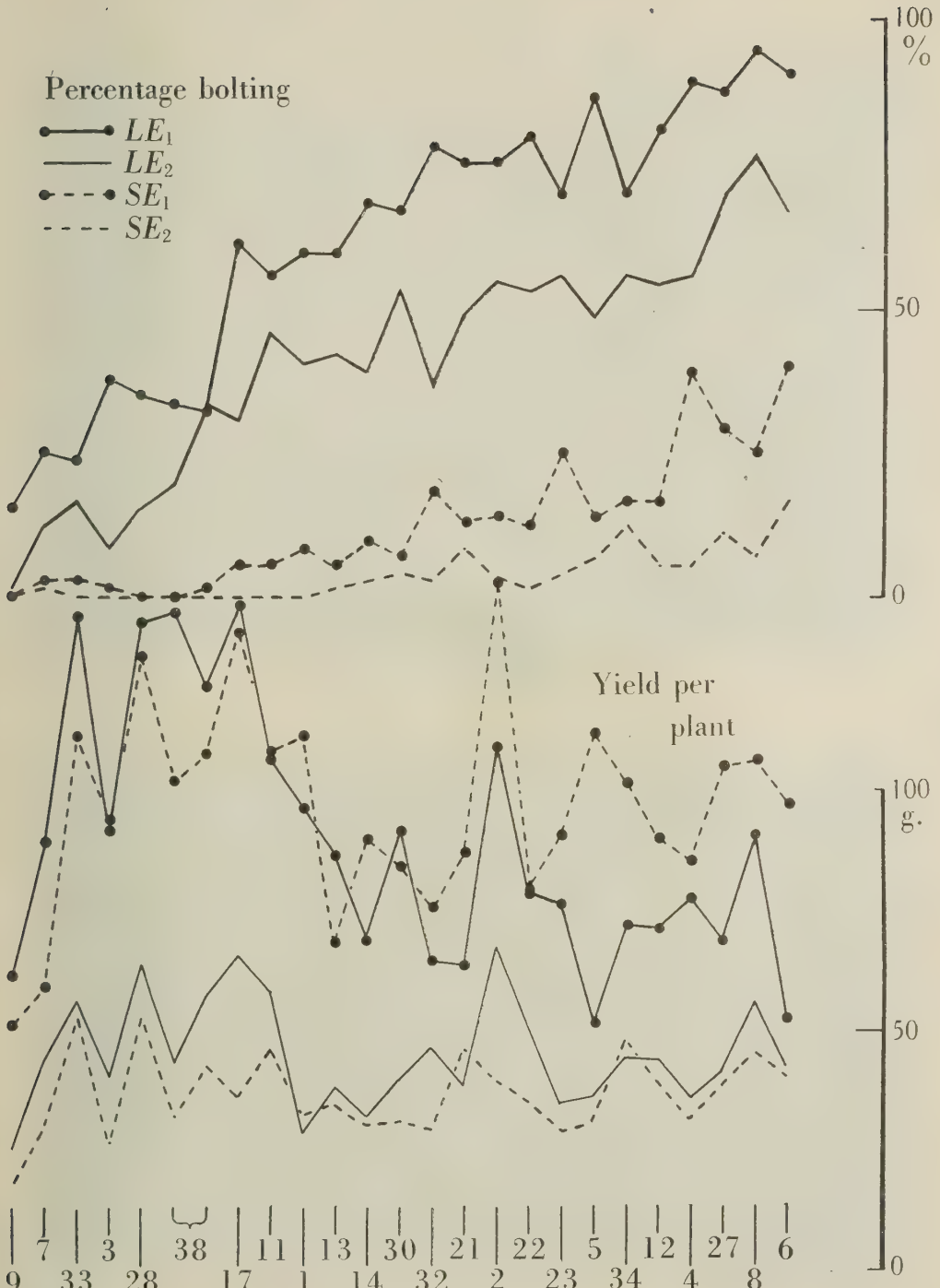
| V | L | S | V | L | S |
|---|---|---|---|---|---|
| 1 | 5·89 | 2·32 | 21 | 5·99 | 2·46 |
| 2 | 6·25 | 2·35 | 22 | 5·27 | 1·93 |
| 3 | 3·71 | 1·60 | 23 | 5·79 | 2·43 |
| 4 | 6·23 | 2·43 | 27 | 5·36 | 2·09 |
| 5 | 5·23 | 2·17 | 28 | 5·41 | 2·03 |
| 6 | 6·07 | 2·56 | 30 | 6·00 | 2·39 |
| 7 | 4·61 | 1·83 | 31 | 5·86 | 2·22 |
| 8 | 5·50 | 2·12 | 32 | 5·84 | 2·26 |
| 9 | 3·49 | 1·46 | 33 | 4·51 | 1·81 |
| 11 | 5·46 | 2·01 | 34 | 6·24 | 2·61 |
| 12 | 5·80 | 2·22 | 38 } | 4·80 | 1·99 |
| 13 | 5·29 | 2·00 | 38′ } | | |
| 14 | 4·51 | 1·79 | | | |

Text-fig. 6 shows these interactions very clearly. In the upper diagram it is seen that the large sets always bolted more than the small, and that within each size class early planted sets always bolted more than late planted sets. On yields (the lower diagram) the effects were, however, very different: within the late planting large and small sets gave approximately equal yields although for some varieties large sets were better than small. The curves for the early planting are seen to cross somewhere between varieties 11 and 30; for varieties bolting less than did V11 it was better to plant large sets, for those bolting more than V30 small were better. For all varieties and sizes it was better to plant early than late. When, however, yield of bulbs which do not contain flower stalks is considered, it is found that with early planting, only in varieties 7 and 9 (which bolted hardly at all, but are useless in practice because their yields were so small) do large sets produce the greater yield. Within the second planting, where the bolting was less, the 'non-bolting' yield of the large sets was greater than that of the small ones for a number of varieties which bolted little.

The bulbs grown from large sets ripened earlier than those from small. This is shown by the count of plants collapsed at the neck on 16 July, for a highly significant difference appears between treatments L and S (52·0 and 44·6 % respectively), and by the counts of plants completely ripe by harvest (57 % for L and 49 % for S).

In the course of the 1942 trial data were obtained for the effect of size on the behaviour of sets in storage. It appears that small sets lose a greater percentage of their weight than do large sets which confirms the findings of Heath (1943*a*). Over the whole experiment the large size class showed an average loss of 2·58 % per 4 weeks, until the time of the first planting, and the small class 3·04 % over the same period, the difference being highly significant statistically. Such a difference would be expected from theoretical considerations, if the loss takes place through the whole surface of the bulb because of the relative mass to surface ratios. Sprouting, on the other hand, was greater for the large sets: 44·9 % of the large sets sprouted by 7 April as against 38·6 % of the small; this difference too, is highly significant.

The author is indebted to Dr O. V. S. Heath of the Research Institute of Plant Physiology for help throughout these experiments especially with the statistical design and with the field work involved; to Miss E. Bruck for help with the field work in the 1940–1 trial and to Dr H. H. Mann for provision of space for the 1942 trial at Woburn Experimental Station, and for supplying labour in that season to cultivate the plots.


Percentage bolting
$LE_1$
$LE_2$
$SE_1$
$SE_2$
100 %
50
0
Yield per plant
100 g.
50
0
9 7 3 38 11 13 30 21 22 5 12 27 6
9 33 28 17 1 14 32 2 23 34 4 8
Varieties

Text-fig. 6.

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## EXPLANATION OF PLATE 2

1941–2 Trial at Woburn Experimental Station, Husborne Crawley, crop season 1942; showing a good (V 38) and a poor (V 6) variety on 6 Aug. 1942.

Fig. 1. The four subplots of V 6 in Square III. From left to right: (1) large sets planted late (14 bolted); (2) small sets planted late (1 plant bolted out of 23); (3) small, early (8 out of 23); (4) large, early (22 out of 24). Compared with fig. 2 the view-point makes the rows appear foreshortened here, but the distances along the rows to the labels of the plots beyond were the same.

Fig. 2. The four subplots of V 38′ in Square II. From left to right the subplots are: (1) small sets planted late (0 plants bolted, out of 24); (2) large sets planted early (14 bolted); (3) small sets planted early (0 bolted); (4) (of the second row only part can be seen) large sets planted late (12 bolted).

Each subplot consists of two rows (that headed by a white label and the row adjacent to the right) in each of which twelve sets were planted. The treatment effects on bolting and on the size of the plants are clearly indicated. Some of the plants—especially in (4) of fig. 1—can be seen to be split and bearing more than one flower stalk.

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# The prevention of seed-borne diseases of flax

# III. The dusting, short wet and fixation methods of seed disinfection in relation to storage of the seed

By John Colhoun, *Plant Disease Division, Ministry of Agriculture, The Queen's University of Belfast*

(With 1 Text-figure)


Flax seed containing up to 10% of moisture was disinfected with a proprietary dry fungicide containing tetramethylthiuram disulphide (Nomersan) at the rate of 12 oz./cwt. of seed, and stored for periods of up to 18 months, without its germination being impaired.

Experiments were made with samples of seed the moisture content of which, before treatment, varied within the range of 5·8–13·2% and which were kept in a commercial store after disinfection. Treatments were carried out with an 8% solution of a soluble organo-mercurial (Ceresan U. 564) at the rates of 0·67 and 0·9 gal./cwt. applied by the short wet method, and with an organo-mercurial powder (Ceresan UT. 1875A) at the rate of 12 oz./cwt. applied by the fixation method using 0·9 gal. of separated milk per cwt. The results obtained show that treatment by either method has the effect of lowering the percentage of viable seeds during subsequent storage and that the higher the moisture content of the seed before treatment the earlier this effect becomes apparent. It is suggested that seed to be treated by the short wet method on a commercial scale should be dried to contain about 5% of moisture, that not more than 0·67 gal./cwt. of liquid be applied and that the seed should not be stored for longer than 3 months after treatment. Following upon these suggestions, 10-ton lots of seed were treated commercially by the short wet method using a Kontramix machine and no ill effects on the crop were observed.

Fig. 1

Fig. 2

Experimental work carried out since 1939 has shown that the seed-borne diseases of flax caused by *Polyspora Lini* Laff., *Colletotrichum Lini* (Westerd.) Toch. and *Botrytis cinerea* Pers. can largely be prevented by seed disinfection (Muskett & Colhoun, 1943, 1944; Colhoun, 1944). Three methods of treatment were found to be effective, but before these could be introduced on a commercial scale it was necessary to study the effects of such treatments on the germination of stored seed.

### Preliminary experiment

A quantity of seed of the variety Liral Crown having a moisture content of 7·2% was mixed thoroughly and 1 cwt. lots were subjected to the treatments stated in Table 1. Treatment with Nomersan by the dusting method was carried out using a hand-operated seed dresser of the type normally used for dressing cereal seeds. In disinfection by the short wet method the seed was spread out on a large non-absorbent sheet, the liquid applied using a watering can with a very fine rose and the seed was then thoroughly mixed. In carrying out treatment by the fixation method the seed was first treated with the requisite amount of powder in the usual manner and then the liquid was applied using the same technique as for the short wet method. The seed was bagged immediately after treatment, but when the short wet or fixation method was employed the sacks were not sewn until 2 hr. after disinfection; one lot of seed was not treated and served as a control. Samples of seed were drawn immediately after treatment, placed in bottles with screw tops and tested in the laboratory for moisture content by drying at 100° C. for 48 hr., the calculation of moisture being on the basis of the undried weight of seed. The results of these tests are given in Table 1, from which it is seen that the short wet or fixation method of treatment increased the moisture content of the seed to a level which is very close to that calculated on the basis of the amount of liquid added. The seed was placed in a commercial store and sampled at intervals to permit of germination tests being made. The germination percentage of each sample on 16 Dec. 1941, 14 months after treatment was carried out, is stated in Table 1. The only treatments which substantially reduced the germination after storage for 14 months were Ceresan U. 564 (8% at 0·9 gal./cwt.) and Ceresan UT. 1875 A (12 oz./cwt. fixed with 0·9 gal. of separated milk).

Table 1. *Effect of seed disinfection on the germination of seed in storage*

| Treatment | Rate per cwt. | Moisture content immediately after treatment % | Germination (after 14 months in storage) (400 seeds tested) % |
|---|---|---|---|
| Untreated | — | 7·2 | 95 |
| Nomersan | 12 oz. | 7·2 | 98 |
| Ceresan U. 564 4% | 0·9 gal. | 13·8 | 97 |
| Ceresan U. 564 8% | 0·45 gal. | 10·6 | 92 |
| Ceresan U. 564 8% | 0·9 gal. | 14·0 | 78 |
| Ceresan 1875 A fixed with separated milk | 12 oz. 0·9 gal. | 13·5 | 89 |

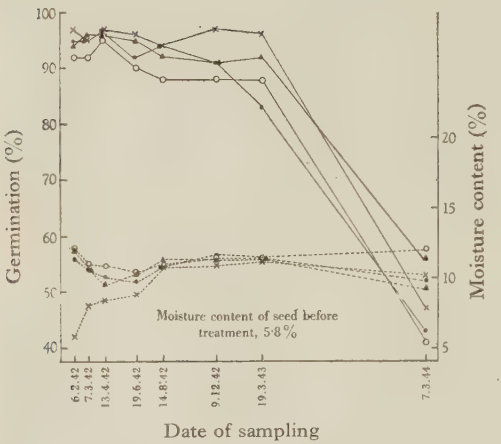
### *Storage of seed treated on a commercial scale with Nomersan*

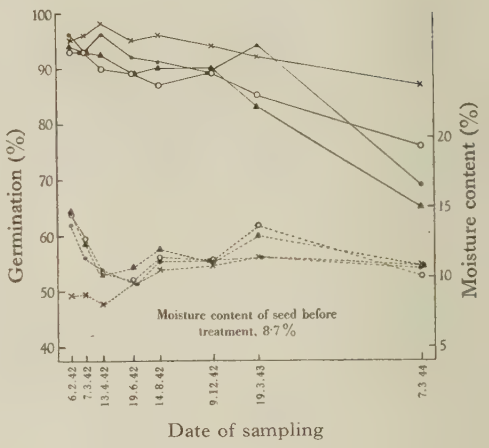
It was decided to disinfect with Nomersan by the dusting method, all sowing flax seed produced in Northern Ireland from the 1940 crop and intended for sowing in 1941. Apart from the treatment of small lots of seed by the short wet method this practice has been continued each season up to the present time. Each lot of seed was examined before treatment to ensure that the moisture content was below 10%, the percentages of germination and purity were determined and each bag was labelled with a distinctive number (Muskett & Colhoun, 1943). In certain years quantities of treated seed were held over in storage, and this afforded an opportunity of studying the effects of treatment with Nomersan on seed stored under commercial conditions. In no case was it found that the viability of the seed was adversely affected as a result of storage for periods of 18 months after treatment.

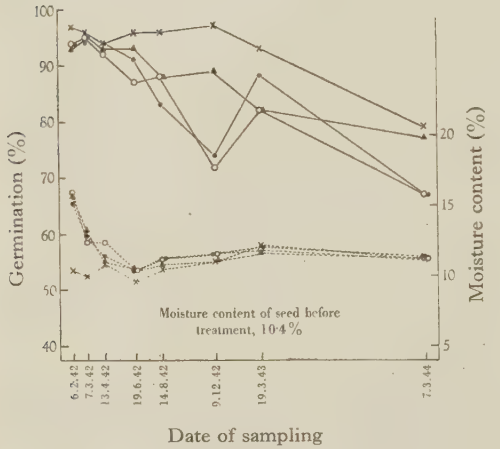
### *Short wet and fixation methods of disinfection in relation to the storage of seed of varying moisture contents*

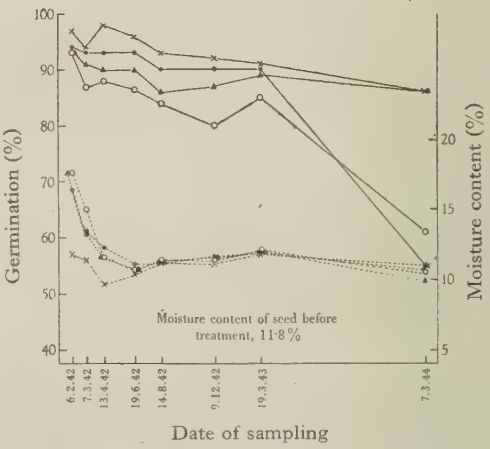
In the preliminary experiment the seed contained 7·2% moisture before treatment, but since the detrimental after-effect of disinfection was found to be associated with the addition of the larger quantity of liquid, it was decided to study the effects of treatment by the short wet and fixation methods when the initial moisture content of the seed varied within a fairly wide range.

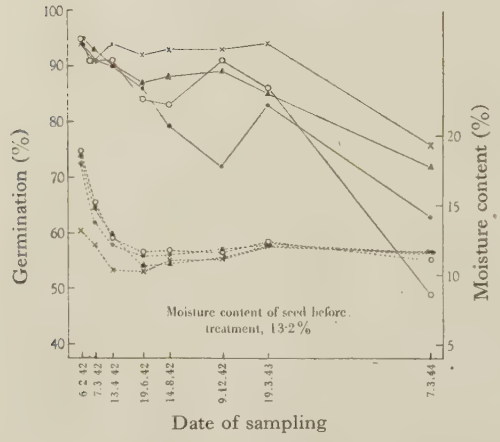
Five lots of seed of the variety Liral Dominion were obtained from the same crop and dried so that after thorough mixing they contained the following percentages of moisture: 5·8, 8·7, 10·4, 11·8 and 13·2 respectively. From each of these lots 1 cwt. was left untreated while similar amounts were submitted to each of the following treatments: Ceresan U. 564 (8% at 0·9 gal.), Ceresan U. 564 (8% at 0·67 gal.) and Ceresan UT. 1875 A (12 oz. fixed with 0·9 gal. of separated milk). In treating seed, the same procedure was followed as in the preliminary experiment. Treatment was carried out on 6–7 Feb. 1942. Samples were drawn within 2 hr. after treatment, the seed was then placed in a commercial store and samples drawn at intervals up to 7 Mar. 1944. The



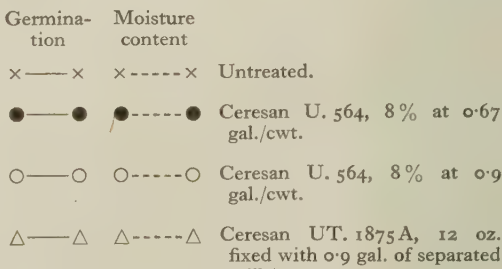




Fig. 1. The viability and moisture content of treated and untreated flax seed during storage.

moisture content and germination capacity of each sample of seed drawn was determined and the results obtained are expressed graphically in Fig. 1.

It is seen from Fig. 1 that after about 6 months in storage the moisture content of each lot of seed, irrespective of the treatment applied or of the initial moisture content, tended to become stabilized in the region of 10–12%. The values obtained for percentage germination show that, on the whole, the untreated seed at all the moisture contents employed continued to germinate well after 13 months in storage, although after a further 12 months the germination percentage in most cases was low. The results show that, in general, treatment has the effect of lowering the percentage of viable seeds when disinfected seed is stored, and that the higher the moisture content of the seed before treatment the earlier this effect becomes apparent. Seed with an initial moisture content of 5·8% treated with Ceresan U. 564 (8% at 0·67 gal./cwt.) did not have its germination seriously impaired until after about 6 months in storage.

*Storage of seed treated by the short wet method on a commercial scale*

In each of the years 1943 and 1944 about 10 tons of seed was disinfected by the short wet method using Ceresan U. 564 (8% at 0·67 gal./cwt.), employing a Kontramix machine for continuous treatment (Muskett, 1944). The moisture content of the seed before treatment was between 5 and 6%. The seed was sown about 3 months after treatment, except for one bag in 1943 which remained in storage for 12 months and which at the end of this period showed a germination of 76% as compared with the initial value of 95%. The brairds resulting from the treated seed sown at the usual rate were very satisfactory and good crops resulted.

## Discussion

Seed containing not more than 10% moisture may be treated with Nomersan at the rate of 12 oz./cwt. by the dusting method and stored for periods of up to 18 months without undergoing deterioration, provided the store is dry. Disinfection may therefore be carried out at any time after the seed has been cleaned.

The position with regard to disinfection by the short wet or fixation methods is not so simple, since these treatments involve increasing the moisture content of the seed. This increase may have an important bearing on the germination of the seed during subsequent storage, for Davin (1928) showed that when flax seed, having a moisture content of 10% of the oven-dry weight (9·1% calculated on the basis of undried weight) or higher, is stored for any length of time it deteriorates very rapidly. The present experiments show that under good commercial conditions of storage there is a tendency for dry seed to absorb, and for damp seed to lose, moisture until the moisture contents of all samples, irrespective of their initial values, become more or less stabilized within a definite range. This range may be expected to vary from store to store depending on such factors as humidity and temperature. It can be expected therefore that the correlation between the moisture content of the seed at the commencement of storage and the germination at any subsequent time will be influenced by the conditions prevailing in the store.

The results presented in this paper show that seed can be treated with safety by the short wet or fixation methods of disinfection, but the germination percentage of the treated seed declines during storage. Apart from the conditions in the store, the length of time during which such treated seed may remain in storage without its viability being impaired is governed by the moisture content of the seed before treatment and the amount of liquid applied. The evidence shows that when the initial moisture content is under 6% and 0·67 gal. of liquid per cwt. is used in treatment, the seed can be stored for periods up to at least 3 months without damage resulting. The results suggest that if the storage conditions are good this period may be extended.

The writer wishes to express his thanks to Dr A. E. Muskett for much valuable advice and criticism; to Messrs R. Stevenson and Son, Ltd., Dungannon, for providing facilities; to the Seed Testing Station, Northern Ireland, for carrying out the germination tests, and to Mr J. P. Malone for photographing the illustrations. Acknowledgement is made to the Flax Development Committee for its generosity in continuing to provide a grant-in-aid to the Plant Disease Division in support of the investigation of diseases of flax.

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# Chemical changes in beech litter due to infection by *Marasmius peronatus* (Bolt.) Fr.

By G. C. M. Harris, *Mycology Laboratory, University Department of Botany, Oxford*

The chemical effect of *Marasmius peronatus* on beech litter is described, with particular reference to the ecological relation between fungus and substrate. Beech leaves, some infected, and others uninfected by this fungus, were analysed and the results compared.

Analysis shows that a vital process involving loss of carbon is at work, and that the fungus decomposes lignin, converting it into soluble products by the action of exoenzymes. It is suggested that by this process it is contributing to conditions favourable for beech regeneration.

The fact that this fungus can more successfully colonize hardwood litter (e.g. beech) than other types of substrate, is explained by consideration of its physiology.

## Introduction

The work here described had two objects: (i) to investigate the chemical effects of a litter-destroying fungus, using methods similar to those employed for wood-destroyers; and (ii) to discover whether such an investigation sheds any light on the ecological relationship existing between fungus and substrate.

It is realized that criticism can be levelled at the use of material which has been subjected to the influence of many uncontrolled factors, but this preliminary examination is offered until more intensive work can be carried out on the effect of a pure culture on freshly fallen litter.

## Analysis of litter

*Marasmius peronatus* is characteristic of, though not exclusive to, beech woods in the deep litter of which it produces a vigorous growth of easily recognizable mycelium.

Uninfected and infected leaves were collected, a year after their deposition, from the deep litter of Saxon entrenchments within a pure beech wood at Hampstead Norris, Berkshire. The uninfected leaves were dark brown, and brittle when dry; the infected ones were straw-coloured, thin and fragile and covered with mycelium. With the exceptions noted, made necessary by the nature of the material and the facilities available, the methods used for the examination of both sets were those, based on Schorger (1926), adopted by the Forest Products Research Laboratory, Princes Risborough, for the analysis of wood.

## Discussion of results

As already noted by Hungate (1940) there is a transfer of nitrogen from leaf to fungus. This applies also to other minerals present in the ash.

Reference to Table 1 indicates that decay has produced an increase in both the cold- and hot-water soluble fractions, which, not being attacked, accumulate. The high value for the alkali solubility of the uninfected material suggests the action of bacteria or micro-fungi or both; but there is evidence (Hilpert & Wagner, 1935) that the skeletal tissue of deciduous leaves consists predominantly of hemicelluloses which would be situated in this fraction. Comparison with the infected litter shows no further increase above that accounted for by increase in hot-water solubility, nor has the fungus attacked the furfuraldehyde-yielding carbohydrates, whether they are associated with the cellulose or not.

Table 1. *Litter analysis (average of several tests)*

| | Uninfected % of sound dry litter | Infected % of sound dry litter | Difference due to decay |
|---|---|---|---|
| (1) Loss in weight | — | 15·5 | +15·5* |
| (2) Ash | 7·4 | 7·4 | None |
| (3) Cold-water soluble | 4·4 | 10·5 | + 6·1* |
| (4) Hot-water soluble | 12·6 | 21·4 | + 8·8* |
| (5) 1 % NaOH soluble | 48·6 | 56·1 | + 7·5* |
| (6) Ether soluble | 1·8 | 2·5 | + 0·7 |
| (7) Alc.-benz. soluble | 2·8 | 3·2 | + 0·4 |
| (8) Furfural | 7·1 | 6·3 | − 0·8 |
| (9) C. and B. cellulose | 14·7 | 14·3 | − 0·4 |
| (10) Furfural in cellulose | 3·5 | 3·3 | − 0·2 |
| (11) Lignin | 49·2 | 25·7 | −23·5* |
| (12) Carbon | 47·5 | 34·7 | −12·8* |
| (13) Nitrogen | 1·6 | 1·5 | − 0·1 |

* Significant difference.

(1): the loss in weight due to infection was estimated by weight per unit area of leaf. (8) and (10): for furfuraldehyde estimation, the distillation method of Pervier & Gortner (1923) was used, with titration technique by Powell & Whittaker (1924). (9): the cellulose content was determined by Heuser & Casseus' (1922) modification of the Cross & Bevan method, using a sintered glass filter.

The cellulose also appears not to have been appreciably affected, though the small proportion of this material compared with that found by Falck (1930),

together with the high alkali solubility mentioned previously and the brittle texture of the uninfected leaves when collected, all point to the possibility of prior infection by a brown rot (cellulose-destroying) complex. This question, however, needs further investigation.

The 'lignin' content of the undecayed leaves is much higher than is usual in timber. Comparable values, however, are given by Falck (1930) and by Hilpert & Wagner (1935). Decomposition by the fungus converts the 'lignin' into soluble products faster than they can be assimilated (lignin loss 24%, total loss in weight 15%), and these products remain, at least temporarily, in the leaves.

Disappearance of carbon accounts for about 80% of the total loss in weight, and this percentage, while indicating vital activity resulting in gaseous products, also suggests a gain in oxygen by other substances which remain *in situ*.

## Conclusions

Arbitrary analyses such as the foregoing only allow tentative and comparative conclusions to be drawn, and further work is necessary on beech litter showing no conspicuous fungus infection. However, it is clear that *Marasmius peronatus* is unique amongst known fungi, in that it can utilize the lignin fraction of the litter as its sole source of energy (Cartwright & Findlay, 1943). This lignin it breaks down to a water- or acid-soluble condition in which it may be attacked by other organisms.

Unincorporated humus, a homogeneous combination of modified lignin and protein (Waksman & Iyer, 1932), is characteristic of 'mor' or 'duff', a soil condition unsuitable for forest regeneration. Falck (1926, 1930) considered that such a condition is associated with the presence of brown rots; white rots, which destroy lignin as well as cellulose, he regarded as a co-factor with animal disturbance, in 'mull' formation. This conclusion is not invalidated by Romell's (1935) observation of hyphae in 'mor' and bacteria in 'mull', since, in the former, mycorrhizal types may predominate, and, in the latter, the white rots would have been rapidly disintegrated and their nitrogen mobilized by worms and larvae. It seems probable that *M. peronatus*, possessing, in common with white rots, the ability to break down lignin and being particularly active in deep litter, may play an important part in the rapid conversion of beech debris to an assimilable form.

The second part of the problem under consideration, i.e. the reasons for the fungus being abundant only on this particular substrate, can be deduced by elimination. Observations in culture by the author show conclusively that not only does its aeration requirement preclude serious exploitation of timber, but also its growth is not so rapid in the presence of the essential oils of conifers, and its competitive value, therefore, not so great. The present work indicates that for favourable growth of this species, a substrate should not necessarily contain much cellulose, but certainly a considerable proportion of unchanged lignin—a condition more nearly approached in litter than in grassland.

It is realized, however, that an accurate assessment of the value of substrate analysis to fungus ecology cannot be made until similar investigations on other litter-destroyers have been carried out.

The author is very grateful for the helpful supervision by Dr W. H. Wilkins of this Department and for the constructive criticism by Mr W. G. Campbell of Forest Products Research Laboratory, Princes Risborough.

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(*Received* 29 *May* 1944)

# Mould deterioration of feeding stuffs in relation to humidity of storage

## Part III. The isolation of mould species from feeding stuffs stored at different humidities

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Isolations were made of mould species actively growing on a variety of feeding stuffs stored at fixed humidities between 100 and 65 % for periods of up to 4 years. The factors influencing the species of moulds isolated were: (1) the relative humidity of the atmosphere; (2) the length of the storage period before the isolations were made; (3) the type and origin of the feeding stuffs from which the isolates were obtained.

A critical humidity existed for each species below which the development of mould spores could not take place. At relative humidities (R.H.) between 100 and 90 % a large variety of moulds were able to develop. Members of the Mucorales and the Fungi Imperfecti were not isolated below 90 % R.H. and, although *Penicillium* spp. flourished between 100 and 85 % R.H., they were not isolated below 75 % R.H. *Aspergillus* spp., on the other hand, were able to develop under conditions of very restricted moisture supply. Some members of the *A. glaucus* group were able to grow at humidities as low as 65–70 %.

In general, the most commonly occurring moulds on the feeding stuffs used in these trials were small ascospored species of the *A. glaucus* group, particularly *A. repens* and *A. ruber*. It is considered that it is these species that will most frequently cause mould damage to feeding stuffs in commercial stores.

### Introduction

The factors controlling mould growth on stored feeding stuffs were outlined in Part I of this series (Snow *et al.* 1944). In these experiments observations were made on six typical feeding stuffs stored at a range of fixed humidities.

Mould development was found to take place on some feeding stuffs exposed to a humidity as low as 65 % after a latent period of over 2 years. Observations showed that at high humidities (100–85 %) a wide range of mould species was able to develop, but that at low humidites (75 % and below) only a few were capable of growth. It seemed desirable, therefore, to investigate more fully the mould species that caused deterioration of these stored products, and the range of humidities at which each was able to develop. Such investigations should provide useful information regarding the moulds that are most common on stored feeding stuffs, and against which control measures might suitably be applied.

### Literature

A survey of the literature showed that little detailed work had been done on the isolation and identification of mould species from stored feeding stuffs. Most authors referred to the occurrence of moulds on stored products in only the very general terms of *Aspergillus* spp. and *Penicillium* spp. McHargue (1920), describing the change in acidity values in maize meal during storage, mentioned the occurrence of *Aspergillus glaucus* and one or two other moulds on maize of 15 % moisture. Thom & LeFevre (1921) found that maize of 13–15 % moisture commonly supported the growth of *A. repens*; when the moisture content was raised to 16 % *A. flavus* could develop, and between 18 and 20 % moisture many different mould species were able to grow. Similar observations were made by Koehler (1938) who gave a critical moisture content of 14·3 % for the development of *A. glaucus* on maize. He found that a species of *Penicillium*, causing 'blue-eye' disease, grew on maize of 16 % moisture and also isolated other species from maize of higher moisture content. A study of the moulds developing in cocoa beans was made by Bunting (1930) who assigned most of the species occurring to the Mucorales or the Aspergilli. Of these, *Aspergillus chevalieri*, and possibly *A. sydowi*, had the lowest moisture requirements of all the forms studied and were able to grow on cocoa beans of 8–9·5 % moisture (equivalent to between 82 and 87 % R.H.).

More comprehensive accounts of the moulds occurring on stored products are given by Smith (1928, 1931) and Galloway (1930, 1935), who examined species causing mildew on textiles. These authors describe a number of species which commonly cause staining of yarns and cloths, and they state that the Aspergilli, especially members of the *A. glaucus* group, have the smallest moisture requirement of all the species examined. In particular, Galloway (1935) studied the germination of the spores of fungi isolated from textiles when exposed on viscose sheeting soaked in dilute wort to given humidities. Spores of members of the *A. glaucus* group, *A. versicolor* and

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*A. candidus* are able to germinate at 75–80% R.H.; other *Aspergillus* spp. require 80–90% R.H.; most *Penicillium* spp. can germinate at 85% R.H.; but members of the Mucorales and the Fungi Imperfecti require humidities of over 90%. It must be pointed out, however, that these observations on spore germination only covered a period of 2 weeks.

## EXPERIMENTAL

### *Method of isolation of mould species*

Isolations were made of the moulds growing on six different feeding stuffs which were stored at a wide range of humidities (Snow *et al.* 1944). Many of the samples had been stored at fixed humidities for as long as 4 years. In addition, isolations were made of moulds developing on samples of other feeding stuffs, including dried grass, palm-kernel cake and groundnut cake, stored for shorter periods at various humidities. The isolations were made by transferring a small piece of growing mycelium with a sterile needle to the centre of a Petri dish containing beer-wort agar. For those samples of feeding stuffs which had supported the development of mould fructifications, a single spore head was transferred with the sterile needle point to an agar plate. In this way it was possible to obtain isolates of the moulds which were actively growing on the feeding stuffs stored at the various humidities. This method was considered to be preferable to any 'plating-out' technique which would have allowed the appearance on the plates of colonies from dormant mould spores present on the feeding stuff but unable to grow at the particular humidity of storage. The Petri dishes were incubated at 25° C., and transfers were made of the developing colonies on to beer-wort or Czapek-Dox agar (20 or 40% sucrose) contained in Petri dishes or in tubes as slopes.

Records were kept of the feeding stuff from which each isolate was made and of the humidity which had allowed the development of the particular mould species. The list of mould species isolated from the feeding stuffs used in these experiments is not regarded as in any way complete. Indeed, the complete isolation of the large number of mould species which are able to develop on feeding stuffs, particularly on those stored at high humidities (100–90%), would prove a never-ending task. Particular attention was therefore paid to the isolation of species whose moisture requirements were low (i.e. species which were able to develop at humidities of 85% or less). It is evident that such moulds are the most likely to be found in the practical storage of feeding stuffs and against which control measures most suitable could be employed. Identifications of the Aspergilli were made with the use of keys provided by Thom & Church (1926) and Thom & Raper (1941) and for the Penicillia by Thom (1930).

### *Factors influencing mould species isolated*

(1) *Humidity of storage.* The isolation of moulds growing on feeding stuffs stored at fixed humidities showed that, while a large number of species were able to develop at humidities of 90–100%, the number of different species isolated from humidities below this figure was limited. It was evident that a critical humidity existed for each species below which the development of mould spores could not take place. Thus, in general, members of the Mucorales and the Fungi Imperfecti were only isolated from humidities of 100–90%; *Penicillium* spp. were not able to grow below 75% R.H. and only flourished at humidities of 85% R.H. and above, but *Aspergillus* spp. were able to develop under conditions of very restricted moisture supply. Members of the *A. glaucus* group were particularly resistant to dry conditions and were able to germinate at humidities as low as 65–70%.

TABLE 1. *Minimum humidities below which the different mould species were not isolated*

| % R.H. | |
|---|---|
| 65 | *Aspergillus echinulatus* (Delacr.) |
| 67 | *A. repens* (Corda) Sacc. |
| 70 | *A. ruber* (Spieckerm. & Bremer). *A. candidus* Link |
| 75 | *A. penicilloides* series. *Paecilomyces varioti* Bain. *Penicillium spinulosum* Thom |
| 80 | *Aspergillus chevalieri* (Mang.). *A. amstelodami* (Mang.) |
| 85 | *A. versicolor* (Vuill.) Tirab. *A. sydowi* (Bain. & Sart.) |
| 90 | *A. niger* series. *Penicillium luteum* series. *P. cyclopium* Westl. *Sporotrichum* sp. *Mucor spinosus* Van Tiegh. |
| 100 | *Penicillium rugulosum* Thom. *Trichoderma* sp. *Rhizopus nigricans* Ehrenb. *Verticillium cinnabari um* (Corda). *Alternaria tenuis* Nees |

Table 1 lists some of the moulds isolated, together with the critical humidity below which isolations of each particular species were not made. This table shows that three members of the *A. glaucus* group (*A. echinulatus*, *A. repens* and *A. ruber*) were able to develop at humidities of 70% and below. Other members of the *A. glaucus* group which were isolated (*A. chevalieri* and *A. amstelodami*) were only isolated at relative humidities of 80% and above. Of the other *Aspergillus* spp. isolated from the feeding stuffs, *A. candidus* was able to develop at humidities of 70% and above, *A. penicilloides* was isolated from samples stored at 75% R.H. and above, *A. versicolor* and *A. sydowi* required a minimum humidity of 85%, but *A. niger* was only isolated from humidities of 90–100%.

Most of the *Penicillium* spp. isolated grew extensively at 90% R.H. and some were able to develop at 75% R.H. Thus, one strain of *P. spinulosum* Thom was isolated from linseed cake and oats samples

stored at 75% R.H. Isolations were also made of *Paecilomyces varioti* (a species closely allied to *Penicillium*) from palm-kernel cake stored at humidities of 75% and above. Species other than *Aspergillus* spp. and *Penicillium* spp. were only isolated from humidities of 90% and above.

(2) *Time of making isolations.* The length of storage before the isolations were made markedly affected the kinds of mould isolated at some humidity levels. At humidities below 80% the number of species able to develop was limited and, as was shown in Part I of this series (Snow *et al.* 1944), these often required long latent periods before spore germination could take place. At humidities above 80%, however, a succession of mould species was able to develop on many of the samples. Thus at 100% R.H. members of the Mucorales quickly established themselves within 2 or 3 days and provided the dominant mould present. After a week, however, members of the *Aspergillus glaucus* group and *Penicillium* spp. had grown rapidly on these samples and provided the dominant moulds at the expense of the Mucorales. *Aspergillus niger* did not appear on these samples until after almost 2 weeks, while the slower growing forms, e.g. *A. candidus*, did not appear until after 3 weeks of storage. Some members of the Fungi Imperfecti, e.g. *Sporotrichum* sp., were not evident in the early part of storage but later provided the dominant moulds on many of the samples stored at high humidities.

It was evident that those species which were well adapted to growing on feeding stuffs, e.g. members of the *Aspergillus glaucus* group and, at 85–100% R.H., *Penicillium* spp., developed at the expense of other species which were not so well adapted. Such competition was probably also influenced by the production of mould by-products from the species that were quickly established which, by their staling effect, prevented the development of slower growing species.

(3) *Type and origin of the feeding stuffs.* The infection of feeding stuffs with mould spores may conceivably take place in three ways: first, infection may occur at the time when the original plant which provides the raw material for the feeding stuff is growing in the field; secondly, infection may take place during processing; and, thirdly, it may occur during the transit or storage of either the raw material or the processed feeding stuff. Galloway (1935) pointed out that the origin of some species of mould fungi (e.g. *Aspergillus niger*) found on manufactured cotton goods could be traced back to the cotton field where they caused diseases of the boll. In a similar way it is probable that some mould species isolated from the feeding stuffs used in these experiments could be traced back to the country from which the original plant constituents came. This is particularly true of imported oilseed cakes manufactured in tropical or subtropical countries. Thus *A. chevalieri* is known to be a common contaminant of foodstuffs from such parts (Bunting, 1930). Those feeding stuffs which are manufactured in this country from oilseeds or other raw materials of imported origin will doubtless be partially sterilized by heat treatment during processing, and the extent to which mould spores are destroyed will depend upon the temperature and duration of the cooking process. Subsequent mould contamination will, however, readily take place from the machinery, workers' clothes, sacks and from the air of the places of storage.

It is possible that different feeding stuffs, providing different types and quantities of nutrients, may affect the developing mould flora. The importance of this factor cannot be assessed from the results of the trials described in this paper because it is not possible to differentiate its effect from those of humidity, other conditions of storage, and the place of origin, three factors which appear to be of greater importance. It may be noted, however, that several isolates of the same mould species were often obtained from a single feeding stuff stored at the range of different humidities. This was true of *Paecilomyces varioti* which was isolated from samples of palm-kernel cake stored at 70, 85, 95 and 100% R.H. This species can therefore be said either to be typical of this particular feeding stuff or typical of the conditions under which it was manufactured and stored.

### *Mould types isolated and their frequency of occurrence*

Table 2 gives details of the different feeding stuffs from which isolates of the various *Aspergillus* spp. and *Penicillium* spp. were made. Members of the *Aspergillus glaucus* group form the majority of these isolates. Only one isolate was made of a large ascospored species (*A. echinulatus**), although the small ascospored species of this group were of frequent occurrence. The rarity of these ascospored forms has been noted by Smith (1931) for cotton goods and also by Thom & Raper (1941). These last authors show that the development of these forms is favoured by low temperatures, and they suggest that more isolations of them might be made if lower incubation temperatures were employed.

Of the small ascospored species (6 $\mu$ or less in long axis) of the *A. glaucus* group, *A. amstelodami* was easily distinguished by the roughened faces of the spores. The colonies of this species were of a dark blue-green colour, brightly speckled with the yellow of the ascocarps.

Several strains of *A. chevalieri* were isolated from different samples of linseed cake. The ascospores of

* Average measurements of the ascospores of this species were 8·8 $\mu$ by 6·4 $\mu$. These are somewhat smaller than the figures given by Thom & Raper (1941) for this species, but the roughened ridges and faces of the ascospores justified its inclusion in this group of the large ascospored forms.

all the strains of this species were typical with their crestlike ridges giving rise to the recognizable pulley shape. The colony character of these strains was, however, very variable. In general, at an incubation temperature of 25° C., the colonies were largely ascocarpic, the yellow colour of the ascocarps predominating over the green shades of the conidia, although different strains of this species showed different ratios and different forms of zonation of the asco-

TABLE 2. *Variety of feeding stuffs from which* Aspergillus *spp. and* Penicillium *spp. were isolated*

| | |
|---|---|
| *Aspergillus glaucus* group | |
| *Aspergillus repens* (Corda) Sacc.<br>Bone meal, bran, dried grass, groundnut cake, linseed cake, locust beans, oats, scotch beans | (67–100 % R.H.) |
| *A. ruber* (Spieckerm. & Bremer)<br>Bran, groundnut cake, linseed cake, locust beans, scotch beans | (70–100 % R.H. |
| *A. chevalieri* (Mang.)<br>Linseed cake | (80–100 % R.H.) |
| *A. amstelodami* (Mang.)<br>Linseed cake | (80–100 % R.H.) |
| *A. echinulatus* (Delacr.)<br>Linseed cake | (65 % R.H. only) |
| Other Aspergilli | |
| *A. candidus* Link<br>Bran, linseed cake, locust beans, oats | (70–100 % R.H.) |
| *A. penicilloides* series<br>Bone meal, oats | (75–100 % R.H.) |
| *A. versicolor* (Vuill.) Tirab.<br>Linseed cake, oats | (85–100 % R.H.) |
| *A. sydowi* (Bain. & Sart.)<br>Linseed cake | (85–100 % R.H.) |
| *A. niger* series<br>Linseed cake | (90–100 % R.H.) |
| Penicillia | |
| *Paecilomyces varioti* Bain.<br>Palm-kernel cake | (75–100 % R.H.) |
| *Penicillium spinulosum* Thom<br>Linseed cake, oats, palm-kernel cake | (75–100 % R.H.) |
| *P. luteum* series<br>Palm-kernel cake | (90–100 % R.H.) |
| *P. cyclopium* Westl.<br>Linseed cake, palm-kernel cake | (90–100 % R.H.) |
| *P. rugulosum* Thom<br>Palm-kernel cake | (100 % R.H.) |

carpic and conidial areas. The growth rate of the strains was also variable; seven different strains of the species gave colonies measuring 7, 11, 14, 20, 26, 33 and 38 mm. diameter when grown on duplicate plates of beer-wort agar incubated at 25° C. for 8 days. In addition, the amount of orange-red colour produced in the medium (given as a character for this species by Thom & Raper (1941) and by other authors) varied with the different strains. The slow-growing strains produced abundant red coloration in the medium while the faster growing strains often showed no colour in the medium.

The most commonly isolated mould species from the feeding stuffs used in these experiments, which were also members of the *A. glaucus* group, were those forms whose ascospores were smooth and in which the equatorial ridges of the furrow were either lacking or only low and rounded. These isolations were assigned to the *A. repens* or the *A. ruber* groups. Ascospores of *A. repens* were identified as being without any well-marked furrow. Those of *A. ruber*, on the other hand, showed a well-marked broad shallow furrow, bounded with low rounded equatorial ridges. For some strains of these two species, however, the definiteness of the furrow did not provide a reliable index for identification; the ascospores of some strains assigned to *A. ruber* were without any definite furrow and some showed only a trace of a furrow. More attention was therefore paid, for these intermediate strains, to colony characteristics. Strains of *A. ruber* could be differentiated by the ruby red pigment produced in the medium, while *A. repens* gave a dirty grey to black coloration in the medium as the colonies developed.

Several strains of *A. repens* were isolated, the majority of them being closely allied in growth characteristics to the type strain. Some, however, showed much slower rates of growth and a sparser development of mycelium and conidia on a beer-wort agar medium than the type strain of this species. Variation was much more marked in the strains of *A. ruber* isolated. The majority of the strains were mainly ascocarpic when grown as colonies on a beer-wort agar medium at 25° C., but the extent of red or orange-red coloration of the mycelium and the ascocarps varied for the different strains. Other strains were isolated whose colonies showed bright emerald conidia intermixed with red ascocarps. One strain isolated was predominantly conidial, ascocarps being produced only in small numbers.

The frequency of occurrence of the small ascospored species of the *A. glaucus* group on such a wide variety of feeding stuffs, particularly *A. repens* which was isolated from humidities ranging from 67 to 100 % R.H., suggests that these species are the best adapted to growth on materials in which the supply of moisture is limited. Mould damage to feeding stuffs in commercial stores will, in the main, be due to these forms. Where conditions of prolonged high humidity (above 85 % R.H.) prevail, other Aspergilli (e.g. *A. sydowi* and *A. versicolor*), Penicillia and other mould species will be able to establish themselves.

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# Certain aspects of resistance of plum trees to bacterial canker

## Part I. Some biochemical characteristics of *Pseudomonas mors-prunorum* (Wormald) and related phytopathogenic bacteria

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Several strains of *Pseudomonas mors-prunorum* (Wormald) and *Ps. prunicola* (Wormald) isolated from pathological lesions of plum and cherry were studied together with the causal organism of bacterial canker of stone-fruits in California (*Ps. syringae* from apricot) and other phytopathogenic bacteria obtained from pear and syringa. Comparison was also made with pseudomonas forms pathogenic to pea, bean, lettuce, and tobacco, and with the common saprophytes *Ps. fluorescens* and *Ps. pyocyaneus*.

With the exception of two yellow organisms (*B. pruni* and the Pear 8 strain—the latter, however, very occasionally showing fluorescence), all belong to the green-fluorescent group of *Pseudomonas* (Dowson's Group II). On the basis of their dissimilation of C and N compounds a very close relationship has been established between these fruit-tree and syringa pathogens of the green-fluorescent group. *Ps. mors-prunorum* is not highly specialized in its nutrient requirements but can satisfy its fundamental C and N requirements from a very large variety of simple substances. The only consistent biochemical differentiation shown by *Ps. mors-prunorum* (including some of the syringa strains) in comparison with *Ps. prunicola* (including *Ps. syringae* from apricot and most of the pear strains) is its more rapid production of acid from sucrose. Both the *mors-prunorum* and *prunicola* varieties produce a levan from sucrose, which causes a raised gummy growth on solid sucrose-containing media. This applies also to *Ps. pisi*, *Ps. tabaci*, and *Ps. phaseolicola*, but is not the case with the weakly pathogenic forms—*Ps. marginalis*, *cerasi* (= *trifoliorum*, from bean), and the saprophytes—*Ps. fluorescens* and *Ps. pyocyaneus*.

On the basis of biochemical characteristics, considered apart from host pathogenicity, there is no justification for erecting to specific rank these various levan-forming, green-fluorescent, phytopathogenic pseudomonads.


### Introduction

Considerable differences in the resistance and susceptibility of varieties of plums to bacterial canker have been reported by Wormald (1934). Before investigating in detail the possible causes for these differences it was considered important to gain information about the nutrition of the causal organism, *Pseudomonas mors-prunorum* (Wormald), especially with reference to its degree of specialization in the use of sources of carbon and nitrogen. Furthermore, as there is controversy in the literature concerning the relationship of *Ps. mors-prunorum* with *Ps. prunicola* (Wormald) the causal organism of shoot-wilt in plums (Wormald, 1930) and various similar pseudomonads isolated from diseases of pear and syringa, it seemed desirable to compare these organisms in

parallel with typical cultures of the pathogen under special investigation.

### Description and origin of cultures

*Ps. mors-prunorum* (Wormald), nine strains, of which five (A61, A63, A64, A65, A66) were newly isolated from stem canker of Purple Pershore plum at the commencement of this work in autumn 1941; A60 was obtained at the same time from a leaf spot on Victoria plum; A59 from cherry canker, 1941; A40 from leaf spot on Victoria plum, 1939; A30 from cherry flower, 1939. All at East Malling.

*Ps. prunicola* (Wormald), three strains: A46 from leaf spot of cherry, 1940; XII*a* from leaf spot of cherry, 1932; XLVI from cherry branch, 1932; East Malling.

*Ps. syringae* (Van Hall) Bergey *et al.*, two strains (Ap1, Ap2) from shoot-wilt and limb canker of apricot, isolated by Dr Wilson at Davis, California, U.S.A., 1942. *Ps. syringae* is considered by him (Wilson, 1931) to be identical with *Ps. prunicola*.

*Ps. fluorescens-liquefaciens* (Spiers), N.C.T.C. 3756, Lister Institute.

*Ps. pyocyaneus*, from Prof. Fleming, through Dr Oxford.

*Ps. pisi*, from pea; *Ps. tabaci*, from tobacco; *Ps. cerasi*, from bean; *Ps. phaseolicola*, from broad bean; *Ps. marginalis*, from lettuce; all from Dr Dowson, Cambridge.

*B. tumefaciens*, two strains, from raspberry, tomato, East Malling.

*B. pruni*, from plum, U.S.A., through Dr Wormald.

Also three unnamed strains from syringa, one from forsythia, seven from pear fruit or flowers—of which Pear 4 was a re-isolation of Pear 3, and Pear 6 of Pear 5; another (Pear 8) originally from Lister Institute, N.C.T.C. 393; all from Dr Wormald, East Malling.

All of these, except *B. tumefaciens*, *B. pruni*, and Pear 8, belong to the gram-negative, lophotrichous, green-fluorescent section of *Pseudomonas* Migula, which produce a white or colourless growth on ordinary solid media, of which the type species is *Ps. fluorescens*—the Group II of Dowson (1939). *B. pruni* and Pear 8 produce a yellow non-diffusible pigment in their growth on solid media, and belong to Dowson's Group III for which he has suggested the generic name *Xanthomonas*. *B. tumefaciens*, with its non-motile variants, is classed with other peritrichous bacteria in the genus *Bacterium* Ehrenberg (Dowson's Group I). According to Dowson, *Ps. marginalis* (or *B. marginatum*) while having polar flagella has the other characteristics of Group I in which it is classed by him, but in the hands of the writer it has shown a pronounced tendency to produce fluorescein and is considered as belonging to Group II. *Ps. pyocyaneus* is the only organism in this list which has been found to produce pyocyanin in addition to fluorescein, but apart from this distinctive characteristic it can be grouped with *Ps. fluorescens*.

### Pathogenicity

In order to check the virulence of the strains, inoculation experiments were made on hosts which are quicker in yielding results than plum trees. Lettuce and tobacco plants, which had been raised in the greenhouse and were kept moist under bell-jars or in a frame during the experiments, were tried but with no results in the case of the lettuces and with only three weakly positive results (unconfirmed) in the case of the tobacco plants. It is noteworthy that *Ps. marginalis*, pathogenic to lettuce, and *Ps. tabaci*, pathogenic to tobacco, were both without effect on their respective hosts under the conditions employed (prick inoculations), and can therefore be presumed weakly virulent or avirulent strains. Clara (1934) reported *Ps. marginalis* to be very weakly pathogenic under experimental conditions.

When immature green plums were used, eight out of the nine *mors-prunorum*, and two out of the three *prunicola* strains caused brownish black lesions on at least one of three occasions, one strain (A40) being consistently virulent, six others (A46, A60, A61, A63, A64, A66) giving positive results in two cases out of three. None of the other named organisms nor the pear and syringa cultures yielded any results on plums.

On dwarf bean pods *Ps. phaseolicola* and *Ps. pisi* caused definite lesions, a positive result which was confirmed. *Ps. cerasi* (=*Ps. trifoliorum*, synonymy suggested by Clara and accepted by Dowson) gave weakly positive reactions on bean pods. A similar weak reaction was yielded by *Ps. marginalis* and *Ps. tabaci*. The majority of the *mors-prunorum*, *prunicola*, apricot, pear, and syringa strains gave good positive results on at least one of three occasions.

Those which were completely negative in their action on all the experimental hosts were the yellow organisms *B. pruni* and Pear 8, the crown-gall organism *B. tumefaciens*, and the two saprophytes *Ps. pyocyaneus* and *Ps. fluorescens*. It may, therefore, be considered that the different green-fluorescent phytopathogenic bacteria under consideration possess in varying degree some property of pathogenicity to plant tissues, and especially so in the case of the several isolates of *Ps. mors-prunorum*.

### Dissimilation of C and N compounds

A simple synthetic medium was employed throughout for testing the availability to the micro-organisms of various classes of C and N compounds. This basal medium contained the following salts:

| | | |
|---|---|---|
| $K_2HPO_4$ | 1·0 g./l. | |
| $MgSO_4.7H_2O$ | 0·5 g./l. | |
| KCl | 0·5 g./l. | |
| $FeSO_4.7H_2O$ | 0·005 g./l. | $p$H = 7·0 |

to which was added 10 g. of the required sugar or other C source, sterilized separately if autoclaving

TABLE 1. *Dissimilation of* C *compounds on microcosmic salt medium*

| C compound | *Ps. mors-prunorum* (9) | *Ps. prunicola* (3) | *Ps. syringae* (2) | Syringa 1–4 | Pear 1–4 and 7 | Pear 5 and 6 | Pear 8 |
|---|---|---|---|---|---|---|---|
| Monosaccharides: | | | | | | | |
| Arabinose | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl | a |
| Xylose | a | a | a | a | a | a | a, y |
| Rhamnose | o | o | o | o | o | o | a |
| Glucose | a, fl | a, fl | a, fl | a | a, fl | a, br | a, y |
| Fructose | a | a | a | a | a | a, br | a |
| Galactose | a, fl | a, fl | a, fl | a (1, fl) | a, fl | a, br | a |
| Mannose | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl |
| Sorbose | o | o | o | o | o | o | o |
| Disaccharides: | | | | | | | |
| Maltose | o | o (1, n) | n | o (1, n) | o (1, n) | n, fl | n, y |
| Sucrose | a, op | a, fl | a, fl | a, op | a, fl | a | a |
| Lactose | o | o | o | o | o | o | n |
| Cellobiose | n (2, o) | n (1, o) | n | o | o (1, n) | n | o |
| Trehalose | b, fl | b, fl | b, fl | b, fl | b, fl | b, fl | n |
| Trisaccharides: | | | | | | | |
| Raffinose | a | a | a | n | a (1, fl) | a | o |
| Polysaccharides: | | | | | | | |
| Cellulose | o | o | o | o | o | o | o |
| Pectin | a | a | a | a | a | a | a |
| Inulin | o | o | o | o | o | o | o |
| Starch | o | o | o | o | o | o | o |
| Glucosides: | | | | | | | |
| Salicin | o (1, n) | o (1, n) | o | o (1, n) | o (1, n) | o | n |
| Polyhydric alcohols: | | | | | | | |
| Glycerol | a, fl | a, fl | a, fl | a (1, n) | a, fl | n | n |
| Dulcitol | o | o | o | o | o | o | o |
| Mannitol | a | a (1, fl) | a | a | a | a, fl | a |
| Aliphatic monobasic acids: | | | | | | | |
| Formic | o | o | o | o | o | o | o |
| Acetic | o | o (1, n) | o | o | o | o | o |
| Butyric | b (2,o) | b (1, o) | n | n (1, o) | b (1, o) | o | o |
| Propionic | o | o | o | o | o | o | o |
| Saturated dibasic acids: | | | | | | | |
| Oxalic | o | o | o | o | o | o | o |
| Malonic | b | b | n | n | b | b | n |
| Succinic | n | b, fl | b, fl | n | b, fl | b, fl | n |
| Unsaturated dibasic acids: | | | | | | | |
| Maleic | n | b, fl | n | n | b, fl | b, fl | n |
| Fumaric | b, fl | b, fl | b, fl | b, fl | b, fl | b, br | b, fl |
| Monohydroxy acids: | | | | | | | |
| Lactic | o | b | b | n | b (1, n) | n | n |
| Malic | b | b | b | b | b | b | b |
| Polybasic hydroxy acids: | | | | | | | |
| Citric | b | b | b | n | b (1, n) | n | n |
| Tartaric | b (2, o) | o | o | o (1, n) | n (1, b) | n | o |

N.B. The figures in brackets indicate the number of strains showing any divergence from the average result listed in each column. The number of strains in each group is given at the top of the columns.

at *p*H 7·0 was liable to cause breakdown of the compound. Organic acids were neutralized by the addition of NaOH. Nitrate-N in the form of 2·0 g. $NaNO_3$/l. was found sufficient with almost all the available C compounds to maintain growth and active motility, but in most cases a more copious growth was yielded by the use of an ammonium salt. Table 1 on the dissimilation of C compounds is compiled from the data obtained from using the above medium with the addition of 4·9 g. sodium ammonium hydrogen phosphate (microcosmic salt). All results were checked at least twice. The cultures were incubated at 25° C. for 20 days, since, although growth usually appeared in 1–2 days, the change in reaction was often slow.

The symbols used to indicate growth and reaction changes are as follows:

a = good growth and production of acid.
b = good growth and production of alkali.
n = sparse growth and approximately neutral reaction.
o = no growth.
fl = green fluorescent pigment.
y = yellow pigment.
br = yellow-brown pigment.
op = milky bluish opalescence.

as regards their fundamental C and N requirements and that such can be obtained from a very large variety of simple substances. *Ps. marginalis*, *Ps. pisi*, *Ps. tabaci*, *Ps. phaseolicola*, *Ps. cerasi*, *Ps. fluorescens*, and *Ps. pyocyaneus* were tested for comparison on substrates representative of the different classes of compounds listed in Tables 1 and 2, and were found to agree very closely in their general biochemical characteristics. They were all capable of growing and, as a rule, of producing pigment on the very simplest media such as aspartic acid only plus the usual inorganic salts. *B. tumefaciens* has more com-

TABLE 2. *Dissimilation of* N *compounds on sucrose medium*

| N compound | *Ps. mors-prunorum* (9) | *Ps. prunicola* (3) | *Ps. syringae* (2) | Syringa 1–4 | Pear 1–4 and 7 | Pear 5 and 6 | Pear 8 |
|---|---|---|---|---|---|---|---|
| Inorganic: | | | | | | | |
| Na nitrate | a, op | b, fl | b, fl | a, op | b, fl | b, fl | n |
| Na nitrite | n | o | o | n | o (1, n) | o | o |
| Ammonium phosphate | a, op | a, fl | a, fl | a, op | a, fl | a, fl | a |
| Na ammonium phosphate | a, op | a, fl | a, fl | a, op | a, fl | a | a |
| Organic: | | | | | | | |
| Ammonium lactate | a | a | a | a | a | a | n |
| Amino acids: | | | | | | | |
| Glycine | b, fl | a, fl | a, fl | a, fl | a, fl | a, br | a, y |
| Alanine | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl | a |
| Arginine | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl | a |
| Phenylalanine | a, op | a, op | a, op | a, op | a, op | a, fl | a |
| Cystine | a | a | a | a | a | a | n |
| Betaine | a, fl | a, fl | a, fl | a (1, fl) | a, fl | a, fl | a |
| Aspartic acid | a, fl | a, fl | a, fl | a, fl | a, fl | a, fl | a |
| Amines: | | | | | | | |
| Glucosamine | a | a | a | a | a | a | n |
| Urea and derivatives: | | | | | | | |
| Urea | b, fl | b, fl | b, fl | b, fl | b, fl | b, fl | n |
| Guanidine | o | o | o | o | o | o | o |
| Uric acid | b, fl (3, n) | n (1, b) | b | n | b, fl | b, fl | n |
| Caffeine | n (2, o) | o | n | n | o (1, n) | o | o |
| Theobromine | n (3, a) | o | o | n | o (1, n) | o | o |
| Glucosides: | | | | | | | |
| Amygdalin | n (4, o) | o | o | o | o (1, n) | o | o |

The same basal salts medium was then used with 1% sucrose as C to test the availability of different N sources, as shown in Table 2.

The amino acids were also tested for their ability to serve as the source of both C and N in the same synthetic basal medium, omitting the sucrose. Arginine, alanine, betaine, and aspartic acid were found capable of so doing for all the strains tested; the same applied to a lesser extent to glucosamine.

Omitting the yellow organism Pear 8, which is differentiated by its particulate growth, insoluble yellow pigment, and ability to grow on rhamnose, it is evident from Tables 1 and 2 that all the green-fluorescent organisms show a very close relationship

plex requirements, as has been shown by the work of McIntire *et al.* (1940). *B. pruni* was found to agree in most respects with the Pear 8 organism.

The present study justifies the inclusion of *Ps. mors-prunorum*, *Ps. prunicola*, *Ps. syringae*, and the other syringa and pear strains in the general definition by Kluyver & Donker (1926) of the genus *Pseudomonas* as heterofermentative bacteria, which, while showing an oxidative dissimilation of the products of protein hydrolysis, are able to use the salts of organic acids, and which are differentiated from the acetic bacteria that share the latter characteristic by their inability to utilize sugars and alcohols well. The proteolytic activities of *Ps. mors-prunorum* and *Ps.*

*prunicola* have already been described by Wormald (1930, 1932), and have been confirmed by the writer for all the strains under consideration. The ability to use the salts of organic acids has been demonstrated by Wilson (1933) in the case of *Ps. syringae*, and by many workers, notably De Jong (1926), in the case of *Ps. fluorescens* and other common saprophytic pseudomonads. In the present instance, the organisms tested showed some differentiation as regards their action on succinic acid, an alkaline reaction accompanied by fluorescence being much more rapidly given by *Ps. prunicola*, *Ps. syringae* (from apricot), and the pear strains than in the case of *Ps. mors-prunorum* and the syringa strains. This was more clearly shown when the less highly buffered nitrate basal medium was substituted for the microcosmic medium, and will be discussed in the succeeding section on pigment production. *Ps. prunicola* and the pear strains also showed better growth and an alkaline reaction with fluorescence on maleic acid. On the other hand, the majority of the *Ps. mors-prunorum* strains were able to utilize tartaric acid which was generally unavailable to the other bacteria.

As regards the utilization of sugars and alcohols, reference to Table 1 shows an acid reaction marked for all the mono- and trisaccharides and the polyhydric alcohols on which growth was maintained. Such a reaction was often slow in appearance and seldom ranged below *p*H 5·5, being often in the neighbourhood of *p*H 6·0 even after 3 weeks' incubation. Large-scale cultures (100 and 200 c.c.) of *Ps. mors-prunorum* on 2·5% glucose-microcosmic medium incubated at 25° C. for 2 and 3 months until no glucose remained have been found to have *p*H 5·0 approximately, and only very small quantities of volatile acid could be detected in the metabolic liquid. This can be regarded as a slow but efficient utilization of the C compound. In the disaccharide group sucrose stands out as the one sugar which affords some basis of discrimination between the strains. Acid production on sucrose is more marked in the case of *Ps. mors-prunorum* and most of the syringa strains than in that of the other organisms, particularly on a nitrate-N medium. Differentiation on the grounds of sucrose dissimilation has been made by Wormald (in a complex organic medium) between *Ps. mors-prunorum* and *prunicola*, by Clara (in a synthetic medium) between weak and strong plant pathogens of the Pseudomonas group, and by Wilson (in a synthetic medium—personal communication, 1942) between different isolates of *Ps. syringae*. There is evidently a consistent variation among phytopathogenic Pseudomonas forms in this respect. Lack of acid production on lactose and salicin (indeed, little or no growth) agrees with Dowson's criteria for his Group II.

In considering the substances widely distributed in plants, the most striking fact that emerges is the inability of these organisms to utilize starch, cellulose, or the other polysaccharides tested except pectin. Soluble pectin (from apple: B.D.H.) may not be completely representative of the complex pectic substances in the middle lamella of cell walls, but it is significant that all the organisms grow very well on it. This fact, however, does not necessarily indicate the secretion of a vigorously acting pectase-enzyme system (cf. Oxford, 1944). There was no evidence of a protopectinase enzyme as shown by softening at the air/liquid interface of strips of potato suspended in culture tubes of the various strains.

### Pigment production and *p*H

Tables 1 and 2 show that all the strains under review are capable of producing the yellow-green fluorescent pigment characteristic of *Ps. fluorescens* on a variety of the C and N sources supplied. Pear 8, on first testing, was thought not to produce the green soluble pigment, but a faint degree of fluorescence was obtained on prolonged cultivation on mannose, salicin, and fumaric acid. Nevertheless, this organism still remains differentiated by its mode of growth and consistent production of an insoluble yellow pigment on solid and some liquid media. Pear 5 and 6 (isolates of the same organism) are placed in a separate column merely to emphasize their tendency in ageing cultures, often accompanied by a drift to the alkaline range, to convert to a dark yellow-brown the normally yellow-green fluorescein. Such a tendency has previously been pointed out for some strains of *Ps. fluorescens* by Topley & Wilson (1929), although it was not shown by the N.C.T.C. strain used in this study.

The media favourable for the production of fluorescein are so, with very few exceptions, for all the strains concerned. This applies also to *Ps. marginalis*, *Ps. pisi*, *Ps. tabaci*, *Ps. phaseolicola*, *Ps. cerasi*, and *Ps. pyocyaneus*. The failure of Wormald (1932) and later of Dowson (1939) to demonstrate the existence of a green coloration on the part of *Ps. mors-prunorum* in nutrient agar and beef extract broth may be attributed to the variability of such complex media; pigmentation occurred on many of the batches of heart-broth agar supplied to the writer, whereas it was absent on the Difco medium used at East Malling. But on synthetic media of known composition fluorescence, wherever it is noted in the tables, could be reproduced consistently by all the nine strains of *Ps. mors-prunorum* (with the frequent exception of A 59), although the precise degree of coloration in a given time often varied with the age and nature of the penultimate culture, and amount of inoculum used.

The green pigment usually appears first in the upper layers of liquid media, then gradually diffuses through the contents of the tube. In the case of media containing sugars from which acid is produced

the pigment may disappear after a shorter or longer period, but on the addition of dilute alkali fluorescence can usually be recovered. This action of acid in suppressing pigment production is more perceptible on a nitrate-N medium than on the more heavily phosphate-buffered microcosmic salt medium. The importance of *p*H in the production of the fluorescent substance has been pointed out for certain plant pathogens by Lacey (1932; in a nutrient broth medium) and Clara (1934; in a synthetic asparagine medium), and for an extensive range of *Ps. fluorescens* strains by Turfitt (1936, 1937; in a synthetic asparagine medium). Turfitt found that while a dilute alkaline solution yielded a green fluorescence, a more concentrated alkaline solution produced a red colour. Such a colour was produced in one instance by an old culture of Pear 6 on a uric acid medium the *p*H of which was over 8. The alkaline range of the green fluorescent pigment has also been confirmed for *Ps. fluorescens* by the work of Turfreijer *et al.* (1938), and, although no chemical investigation has been made of the pigment produced by the present phytopathogenic bacteria, the cultural evidence strongly supports its identity with the fluorescein of the common saprophyte, *Ps. fluorescens*.

The only consistent difference in pigment production between *Ps. mors-prunorum* and the other organisms is that obtaining on different sucrose media. In the diagnostic criteria given by Wormald a greater degree of opacity has been described for *Ps. mors-prunorum* as compared with *Ps. prunicola* on a 5% sucrose-nutrient broth medium. This differentiation has been found to obtain in synthetic sucrose media with inorganic sources of N (see Table 2), the *mors-prunorum* strains (and also the syringa cultures which are very closely allied) showing a milky bluish opalescence in contrast to the varying shades of blue-green-yellow fluorescence given by the *prunicola*, pear, and *Ps. syringae* (apricot) cultures. Such opalescence was associated with a more acid reaction (*p*H 5·0 as against *p*H 8·0 in the green cultures; 3 weeks' incubation on 1% sucrose-Na nitrate). A similar colour differentiation was obtained on a 1% succinic acid-Na nitrate medium with corresponding though not as marked acid and alkaline reactions, but towards the end of the third week fluorescence gradually appeared in the *mors-prunorum* and syringa cultures and the *p*H in all cases finally reached 8·0. The distinction thus appears to be that the *Ps. prunicola* varieties are more easily and quickly able to attack the N source with the production of an alkaline reaction favourable for the production of fluorescence, while the *Ps. mors-prunorum* type in the presence of sucrose produces more acid.

### Synthesis of bacterial polysaccharide and longevity on solid sucrose media

Aderhold & Ruhland (1907) noted the production of a gum by their *B. spongiosus*, the causal organism of bacterial canker of cherry, from sucrose and raffinose only and not from any other sugar nor from a mixture of glucose and fructose, and hazarded the suggestion that it was possibly only the fructose group that served as the source of the polysaccharide which, however, on inadequate chemical evidence they described as an araban. LaGrange (1926) reported the production of a gum from sucrose by an organism designated *Ps. gummosa*. The work of Cooper & Preston (1935) established the fact that with all the phytopathogenic bacteria which they tested (including *B. pruni*, *Ps. prunicola*) the polysaccharide produced from sucrose and raffinose only was a levan, and that the relationship between substrate and synthesized carbohydrate was due to the necessity of there being a source of fructo-furanose available before a levan could be formed. This was confirmed by Lyne *et al.* (1940). The statement by Norman (1937) that levan-forming organisms produce no polysaccharide if supplied with inorganic N has not been borne out by the present work on *Ps. mors-prunorum*, since both the nitrate and ammonium salt media commonly employed have yielded polysaccharide in fair quantity. A sample of this crude substance obtained by alcohol precipitation was sent to Dr M. Stacey, who kindly examined it and reported on the purified specimen that it has 'N 0·3%, ash 3%, $[\alpha]_D - 43°$ (*c.* 1·0) in $H_2O$. It is readily hydrolysed with dilute acids giving a final rotation $[\alpha]_D - 90°$ and the sole product of hydrolysis is fructose. Thus the polysaccharide is a levan.'

It was found that there was a marked decrease in the yield of polysaccharide when cultures of *Ps. mors-prunorum* were incubated for prolonged periods (142 mg. crude substance from 100 c.c. of 5% sucrose-microcosmic salt medium incubated for 6 weeks, as compared with 676 mg. from the same amount maintained for 1 week). This indicates that there may be a reversal of the reaction, the bacteria breaking down the synthesized product as the sugar supply becomes low. The organism in the flask was still viable after 6 weeks. This ability of the bacterium to utilize its own polysaccharide was confirmed by the development of a sparse growth on agar plates containing the polysaccharide as sole source of C, and subcultures were successfully made from this growth. The Californian strains of *Ps. syringae* are even more rapid in breaking down the polysaccharide produced, only an inappreciable quantity being precipitated by alcohol after 10 days' growth, while an abundant yield was obtained after 5 days.

The production of this gummy polysaccharide from sucrose appears to be correlated with the characteristic heaped-up, radially striated, more or less translucent, moist aspect of the colonies on nutrient or inorganic N-agar to which sucrose has been added, whereas on ordinary nutrient agar or solid media containing other C sources the growth is conspicuously flatter and smoother. This applies not

only to *Ps. mors-prunorum*, *Ps. prunicola*, *Ps. syringae*, the syringa, and to a varying and lesser degree some of the pear strains, but also to *Ps. pisi*, *Ps. phaseolicola*, and *Ps. tabaci*. On the other hand, *Ps. fluorescens*, *Ps. pyocyaneus*, *Ps. cerasi*, *Ps. marginalis*, and Pear 8 never produced a raised gummy growth on 5 % sucrose agar media which they coloured green (the addition of 5 % sucrose to solid media in the case of the other organisms inhibited fluorescence), and they failed to yield a polysaccharide in synthetic sucrose liquid media. No discontinuous levan coacervate, such as has been described by Hestrin *et al.* (1943) for *B. subtilis* on sucrose-agar, was noted with any of these organisms. Also, when tested by their method of selective diffusion in agar, the enzyme appeared to be endocellular. But in liquid sucrose media the polysaccharide produced by *Ps. mors-prunorum* could be precipitated from the sterile Seitz filtrate.

In view of the diagnostic importance attached by Wormald to the more rapid dying out of *Ps. mors-prunorum* as compared with *Ps. prunicola* on 5 % sucrose-nutrient agar, a phenomenon already noted by Aderhold & Ruhland for their very similar *B. spongiosus*, the effect of sucrose on the longevity of the *mors-prunorum* and *prunicola* varieties was tested in different solid media. In the first place, it should be mentioned that, although on the Difco nutrient agar plus 5 % sucrose used at East Malling, almost all the *mors-prunorum* strains died out in 4–6 days as stated by Wormald, on the heart-broth agar plus 5 % sucrose later used by the writer viability was frequently extended to 10–12 days when a disintegration of the raised colonies and greatly increased viscosity of the growth usually indicated death. The fact also that sucrose, and to a lesser extent raffinose, is the only sugar from which a levan is produced by both the *mors-prunorum* and *prunicola* varieties, and that 'in the natural or "aggregated" state wide differences do exist in levans from various sources' (Dr M. Stacey), made it a matter of interest to discover whether the polysaccharide played any part in this decreased longevity.

Accordingly, both *Ps. mors-prunorum* (strain A61) and *Ps. prunicola* (A46) were grown on the following media from which subcultures were made at frequent intervals for viability:

(*a*) nutrient heart-broth agar + 5 % sucrose;
(*b*) nutrient heart-broth agar + 5 % sucrose + 1 % Ca carbonate;
(*c*) nutrient heart-broth agar + 5 % raffinose;
(*d*) nutrient heart-broth agar + 5 % glucose;
(*e*) nutrient heart-broth agar + 5 % fructose;
(*f*) nutrient heart-broth agar + 2·5 % fructose + 2·5 % glucose.

All were adjusted to *p*H 7·0, and brom-cresol purple added as indicator. Control media without sugar were also sown.

The characteristic raised, translucent growth was given by both varieties on (*a*), (*b*), and to a less extent (*c*). The addition of the Ca carbonate in (*b*) did not appear to cause any diminution of polysaccharide production, but the neutralization of the acid reaction resulted in the appearance of the fluorescent pigment (which was absent in (*a*)) first in *prunicola* and later in *mors-prunorum* in control tubes from which the indicator was lacking. The latter organism produced an acid reaction (*p*H 5·0) on (*a*) within 2–4 days, and this was the only medium on which longevity was decreased for *Ps. mors-prunorum* (8–12 days) as against 20–30 days on other media and for *Ps. prunicola* (20–30 days) on all. The glucose media gave poor growth.

Another set of media was prepared containing the same sugars in the same proportions added to the synthetic microcosmic salt medium used in the preceding sections. Again, a gummy, raised growth with radial lines occurred on sucrose, sucrose + Ca carbonate, and, this time in the case of *mors-prunorum* only, on raffinose. The growth on the other sugars was smooth and flat. The acid reaction was more marked on sucrose with *mors-prunorum*. No pigment was produced in any case. Both varieties were still viable on all media after 30 days.

This shows that the production of polysaccharide from sucrose has no effect upon longevity, and also that the acid produced from sucrose in an inorganic ammonium salt medium is not toxic. To test whether it was the peptone in the nutrient agar which in combination with sucrose caused the more rapid dying out of *Ps. mors-prunorum*, a similar set of media was made up using 1 % peptone (B.D.H.) in place of microcosmic salt added to the usual inorganic salts. Brom-cresol green was added as indicator, and *p*H 4·0 was noted in 3 days in the case of *Ps. mors-prunorum* on the sucrose medium. However, both organisms were viable after 20 days on all media. It would seem, therefore, that the decreased longevity of *Ps. mors-prunorum* on sucrose-nutrient agar is due not to *p*H, nor to the acid ions produced by the organism from sucrose in a simple inorganic or peptone medium, but probably to the by-products arising in the more complex nutrient medium.

### Production of indole and β-indoleacetic acid

The green-fluorescent bacteria have usually been reported as indole-negative. Peptone-water cultures tested after 4 days by Ehrlich's method all gave negative reactions with the organisms of this study except in the case of Syringa 3 (originally an American strain). When the same cultures were tested by Salkowski's method, a positive reaction was given by *Ps. prunicola* (2 of 3), *Ps. mors-prunorum* (2 of 5), 3 pear strains and Syringa 3. Frieber (1922), testing a number of Gram-negative, non-indole-forming bacteria which showed a positive Salkowski reaction, deduced from experiments with a variety of indole

derivatives that this positive reaction in the absence of an Ehrlich reaction was due to the production of β-indoleacetic acid from tryptophane. Several of the *mors-prunorum*, *prunicola*, pear, and syringa strains were accordingly grown on a glucose-tryptophane medium, and in all cases produced a good Salkowski reaction. It was not produced by growth on tyrosine, arginine, glycine, or alanine.

The production of a growth-substance of auxin type has been noted for several classes of bacteria by different investigators. Rusnizina (1938) found that a number of the *fluorescens* type isolated from soil gave good positive reactions when estimated by the Avena curvature technique, and Roberts & Roberts (1939) state that 66% of the 150 species of actinomycetes, bacteria, and moulds which they obtained from soil produced auxin on organic media. But the lack of any pathological lesion or enhanced cambial activity arising from the injection into plum trees of cell-free filtrates of the tryptophane cultures of *Ps. mors-prunorum* incline the writer to the belief, expressed by Riker *et al.* (1941) in discussing the effect of such growth-substances on the crown-gall disease, that there is as yet no clear evidence that the production of auxin by bacteria is connected with their pathogenicity.

### Inhibiting action of certain classes of compounds on *Ps. mors-prunorum*

The A61 strain of *Ps. mors-prunorum*, recently reisolated from passage in the tree and of vigorous growth, was employed as test organism against the following substances, many of which occur in plant tissues, in both synthetic nitrate-N and broth media. The figures in brackets give the limiting dilution for complete inhibition of growth as read after 48 hr. at 25° C., taking the synthetic series; on broth it was often one tube lower. All acids were neutralized to avoid the effect of the H-ion:

*Aliphatic monobasic acids:* formic (0), acetic (1:100), propionic (1:50).

*Aliphatic aldehydes:* formaldehyde (1:10,000).

*Saturated dibasic acids:* oxalic (1:50).

*Monohydroxy and keto acids:* lactic (1:100), pyruvic (1:500).

*Aromatic acids:* salicylic (0), benzoic (1:400), gallic (1:1000).

*Phenols:* phenol (1:3000), resorcinol (1:2700), catechol (1:3000), hydroquinone (1:2000), pyrogallol (1:3000), phloroglucinol (1:2000).

*Tannins:* tannic acid (1:500).

*Glucosides:* amygdalin (0), arbutin (0), phloridzin (0), salicin (1:50), saponin (0).

*Crude polysaccharides:* plum gum (0), metabolic product of *Ps. mors-prunorum* (0). (5% tested in a solid medium.)

It is evident that the organic acids can be disregarded as inhibiting agents except in so far as they may alter the reaction of naturally available substrates in the host, and that the phenols are the most potent of the substances tested. Catechol, hydroquinone, and phloroglucinol are known to occur in the free state in plants (Onslow, 1929). The action of catechol and pyrogallol may be linked up with the action of tannins, and it will be noted that the gallic acid has an upper limit of 1:1000. The well-known antiseptic properties of the phenols are of further interest in connexion with the phenolic glucosides which here are shown to be without effect on the organism, and it would seem that the addition of the sugar molecule robs the aglucone of any bactericidal power it might possess. Prunasin, isolated from wild cherry bark, was not obtainable, but, since it is a mandelonitrile glucoside, mandelic acid was tried—again with a negative result. However, due attention should be paid to the suggestions of Armstrong & Armstrong (1931) that 'since any particular glycoside is only hydrolysed by its specific enzyme, the supply of these materials for whatever purpose they are required is regulated by a very sensitive control', and that, given such hydrolysis and liberation of the phenolic constituents, 'the universal presence of glycosides in the bark of plants may be. . . explained: they ensure an antiseptic treatment of all wounds in the integument'.

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# The suppression of one plant virus by another

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(With Plate 3)

Severe etch virus prevents the multiplication of potato virus *Y* and *Hyoscyamus* virus 3 and replaces them even in plants in which they are established. Mild etch virus reduces the concentration of potato virus *Y* but does not suppress it completely. Cucumber virus 1 multiplies normally in mixed infections with any of the three other insect-transmitted viruses. Possible implications of these results on the mechanism of virus multiplication are discussed; it is suggested that these viruses inactivate in cell sap at approximately the same rate as they denature *in vitro*.

No differences were found between the stability of antibodies to viruses with different properties.

## Introduction

Two kinds of interaction between pairs of viruses infecting the same plant, one synergistic and the other antagonistic, are well established. The synergistic was the first to be described; the interaction was recognized from the discovery that certain common diseases are caused by simultaneous infection with two viruses neither of which produces such symptoms when present alone. The best known examples of such diseases are probably potato crinkle caused by potato viruses *A* and *X* (Murphy & McKay, 1932) and tomato streak caused by tobacco mosaic virus and potato virus *X* (Vanterpool, 1926). As far as is known, the individuals of such pairs of virus occur in the same concentration in plants infected with one as in plants infected with both. The interaction shows solely in the production of a new type of symptom in plants infected with both, and this

symptom results whether the two viruses enter simultaneously or either enters before the other.

The antagonistic type of interaction has been more widely studied and is believed to be characteristic of the behaviour of related virus strains. Such strains mutually interfere with each others' multiplication and if a plant is fully infected with one strain it resists infection with another. If two strains causing different symptoms enter a plant simultaneously, unless one strain multiplies or moves more rapidly than the other, the resulting symptoms are usually intermediate between those caused by the two strains acting separately.

The possibility of a third type of interaction, the actual suppression of one virus by another, was suggested by work on plants infected with potato virus *Y*, *Hyoscyamus* virus 3 and severe etch virus (Bawden & Kassanis, 1941). Plants infected with virus *Y* were found to be susceptible to infection with the other two viruses. After such secondary infections the plants developed symptoms characteristic of infection with the second virus alone, and serological tests showed that the content of virus *Y* in such plants was reduced below the level necessary to give a visible precipitate with its antiserum. Whether virus *Y* was entirely suppressed by the other viruses or merely reduced in quantity, could not be determined from these tests. The present paper describes further experiments to investigate this point using a wider range of viruses and virus strains and more sensitive methods of testing for the presence of potato virus *Y*.

## Material and methods

The viruses used were two strains of tobacco etch virus, severe etch virus (S.E.V.) and mild etch virus (M.E.V.); potato virus *Y* (P.V. *Y*); *Hyoscyamus* virus 3 (Hy.V. 3) and a strain of cucumber virus 1 (C.V. 1) causing a mild mottle in tobacco. All these viruses are transmitted by *Myzus persicae* Sulz., and have similar relationships with their insect vector (Watson & Roberts, 1939), are readily transmitted by inoculation and have similar properties *in vitro*.

Potato is not susceptible to infection with Hy.V. 3 (Hamilton, 1932), so that specific tests for the presence of P.V. *Y* in plants inoculated with the two viruses can be made by means of transmissions to potato. Majestic, President and King Edward potato varieties were used and transmissions were made with *M. persicae* and by inoculation of sap. In all tests with *M. persicae*, the aphides were starved for 4 hr. before use, fed for only 2 min. on the source of infection and then immediately transferred to the healthy test plants, on which they were left for 24 hr.

The same methods were also used for testing for P.V. *Y* in the presence of S.E.V. and M.E.V., for although potatoes are susceptible to the etch viruses when transmitted by grafting, they are resistant to infection by sap inoculation or by *M. persicae*. From more than fifty potatoes inoculated with S.E.V., only one became infected; this one showed symptoms identical with those of grafted plants. In some respects the symptoms resemble those caused by P.V. *Y*, but the two are readily distinguished. President plants in the first year of infection show a blotchy type of yellow mottling on the upper leaves, which are waved and slightly crinkled. The lower leaves do not shrivel and fall, as when infected with P.V. *Y*, but they show black necrotic streaks running along the underside of the veins (Pl. 3, figs. 1, 2). Infected leaves contain large numbers of intranuclear inclusions, similar to those described in tobacco (Kassanis, 1939). No inclusions were found in tubers from infected plants. Such tubers gave rise to stunted, chlorotic plants with small deformed leaves. M.E.V. produced symptoms that were less severe but of the same general type. Of the viruses used, only C.V. 1 infects cucumber and specific tests for the presence of this virus were made by inoculating sap to cucumber seedlings.

Serological tests were made on sap clarified by centrifuging for 10 min. at 12,000 rev./min. as previously described (Bawden & Kassanis, 1941). The antisera used were all prepared by Dr A. Kleczkowski.

## Interactions between tobacco etch viruses, potato virus *Y* and Hyoscyamus virus 3

The results of all our experiments with S.E.V. and P.V. *Y* suggest that P.V. *Y* is unable to exist in cells that are also infected with S.E.V. Tobacco plants have been inoculated simultaneously with the two viruses, and plants infected with one have been reinoculated with the other. From leaves fully infected with S.E.V. and showing typical symptoms, we could not isolate P.V. *Y* either by using aphides or by inoculating potatoes with expressed undiluted sap. The latter fact indicates that if P.V. *Y* is not completely suppressed by S.E.V., it is reduced to less than 1/100 of the amount in plants infected with P.V. *Y* alone, for sap from such plants usually gives infection in potatoes at dilutions up to 1/250 and sometimes higher.

In only two kinds of leaf from plants infected with both S.E.V. and P.V. *Y* have we been able to find P.V. *Y*, viz. leaves that have been rubbed with inoculum containing both viruses and the lower leaves of plants infected with P.V. *Y* when young and reinoculated with S.E.V. when mature. The results in Table 1 show this persistence of P.V. *Y* in leaves rubbed with both viruses and also show that the quantity of P.V. *Y* is much less than in leaves rubbed with P.V. *Y* alone. Presumably this occurs because during inoculation some cells become infected with P.V. *Y* alone, and as there is little lateral diffusion of viruses from cell to cell in such leaves these cells remain free from S.E.V. This absence of

lateral spread in such leaves is supported by the fact that their virus content, in contrast with that of leaves infected by systemic spread, depends on the concentration of the inoculum. The persistence of P.V. *Y* in the old leaves of plants reinoculated with S.E.V. when mature is simply explained by the fact that S.E.V. does not spread into such leaves. The upper leaves from such plants seem to contain S.E.V. only, whereas the lower contain only P.V. *Y*.

M.E.V. occurs in infected tobacco plants in much lower concentrations than S.E.V. If plants are infected with the two simultaneously, S.E.V. dominates and the plants are indistinguishable from those inoculated with S.E.V. Plants systemically infected with M.E.V., however, resist infection with S.E.V., and if reinoculated with S.E.V. they develop no further

TABLE 1. *Transmission by* M. persicae *from plants inoculated with severe etch virus and potato virus Y*

| Infective source | Tobacco plants infected with S.E.V. | Tobacco plants infected with P.V. *Y* | Potatoes infected with P.V. *Y* |
|---|---|---|---|
| Leaves rubbed with S.E.V. and P.V. *Y* | *5/20 | 0/20 | *1/10 |
| Systemically infected leaves from plants inoculated with S.E.V. and P.V. *Y* | 6/20 | 0/20 | 0/10 |
| Leaves rubbed with P.V. *Y* alone | — | 5/20 | 7/10 |

* The denominator is the number of test plants used and the numerator the number that became infected. Single aphides were used in the transmission to tobacco and five aphides per plant for transmission to potato.

symptoms and there is no increase in the virus content of expressed sap.

The results of infecting plants with both M.E.V. and P.V. *Y* do not duplicate those obtained with S.E.V. and P.V. *Y*, for M.E.V. is unable completely to protect plants from P.V. *Y* or completely to suppress P.V. *Y* in plants in which it is established. The symptoms caused by M.E.V. in tobacco plants somewhat resemble those caused by P.V. *Y* and there is little difference in external symptoms between plants inoculated with either or both. The characteristic nuclear inclusions produced by M.E.V., however, are as abundant in plants infected with both as in plants infected with only M.E.V. Serological tests show that the concentration of P.V. *Y* in plants infected with both is less than that in plants infected with P.V. *Y* alone, for sap from the former fails to precipitate with antiserum to P.V. *Y*.

When tests for P.V. *Y* are made by inoculating diluted sap to potatoes, it is clear that this virus is not completely suppressed as it is by S.E.V., but the content of P.V. *Y* is greatly reduced, the extent of the reduction depending on the order in which the two viruses entered the host plants. This is shown in Table 2, from which it will be seen that the concentration of P.V. *Y* was least in the sap of plants first infected with M.E.V. and then reinoculated with P.V. *Y*.

The effect of the etch viruses on Hy.V. 3 seems to be much the same as on P.V. *Y*. Plants simultaneously inoculated with S.E.V. and Hy.V. 3 develop typical symptoms of severe etch, and sap from such plants precipitates with S.E.V. antiserum, but not with Hy.V. 3 antiserum. Similarly, plants already infected with Hy.V. 3 develop symptoms of severe etch when reinoculated with S.E.V. and their sap ceases to precipitate with Hy.V. 3 antiserum. From leaves of such plants infected by the systemic spread of viruses we have been unable to isolate Hy.V. 3 by means of aphides. From the leaves actually rubbed with both viruses, however, we have recovered Hy.V. 3 by means of aphides, although much less frequently than from leaves rubbed with Hy.V. 3 alone.

TABLE 2. *The reduction of potato virus Y by mild etch virus*

| Source of sap | Range of dilution end-points of sap in different experiments giving infection in potatoes |
|---|---|
| Plants infected with P.V. *Y* alone | 1/100–1/625 |
| Plants first infected with P.V. *Y* and reinoculated with M.E.V. | 1/5 –1/25 |
| Plants inoculated with P.V. *Y* and M.E.V. simultaneously | 1/1 –1/5 |
| Plants first infected with M.E.V. and reinoculated with P.V. *Y* | 1/1 |

M.E.V. has much less effect than S.E.V. on Hy.V. 3. Plants suffering from mild etch are still susceptible to Hy.V. 3, and both viruses can enter and survive together. The external symptoms of plants infected with both viruses resemble those caused by *Hyoscyamus* virus alone, but the presence of M.E.V. is demonstrated by the occurrence of intranuclear inclusions. The content of Hy.V. 3 is less affected than that of P.V. *Y* by the presence of M.E.V.; it is reduced to about one-quarter of the amount in plants infected with Hy.V. 3 alone but is usually sufficient to precipitate with antiserum.

Similarly, Hy.V. 3 itself reduces the concentration of P.V. *Y* but does not suppress it completely. Sap from plants infected with both viruses precipitates fully with Hy.V. 3 antiserum but does not precipitate with P.V. *Y* antiserum. Inoculation of such sap to potatoes, however, shows the presence of P.V. *Y* and from the dilution end-points obtained it would seem that the concentration of P.V. *Y* is about 1/20 of that in sap from plants infected with P.V. *Y* alone.

### Complex diseases with cucumber virus 1

C.V. 1 has many properties in common with S.E.V., Hy.V. 3 and P.V. *Y*; it has similar relationships with its vectors and its resistance in expressed sap towards ageing and heating is identical (Watson & Roberts, 1939). Indeed, Chester (1935, 1937) claims that C.V. 1 and P.V. *Y* are serologically related and so strains of the same virus. We have been unable to confirm this; we have tested a number of sources of C.V. 1 against antiserum to P.V. *Y*, but have never obtained any specific precipitation. We have injected rabbits with C.V. 1 from three different sources, but have failed to produce an antiserum that precipitated sap from plants infected with C.V. 1. The most likely explanation of this is that the virus content of sap from plants infected with our sources of C.V. 1 was too small to give a visible precipitation, as with M.E.V. (Bawden & Kassanis, 1941), and not that the virus did not stimulate the production of antibodies. The sera from these rabbits failed to precipitate sap from plants infected with P.V. *Y* when tested in conditions in which antisera against P.V. *Y* gave precipitation. This cannot be taken as proof that C.V. 1 and P.V. *Y* are serologically unrelated, for in the absence of a reaction with C.V. 1 it is possible that the sera used contained no antibodies to the virus. Taken in conjunction with the results described below, however, it seems unlikely that the two are related and more probable that the virus-sources used by Chester for immunization contained both C.V. 1 and P.V. *Y*.

Tobacco plants infected with C.V. 1 are susceptible to S.E.V., Hy.V. 3 and P.V. *Y*, and the typical vein-clearing symptoms appear at the same time after inoculation as in healthy control plants; similarly, plants infected with any of these viruses are susceptible to C.V. 1. Plants infected with C.V. 1 and with any of the other three viruses show symptoms of a different type and of greater severity than either virus causes alone. This effect is most clearly shown by mixtures of C.V. 1 and P.V. *Y* for neither of these alone produces a severe disease in tobacco (Pl. 3, figs. 3, 5). When the two viruses are present together, however, the infected plants are greatly stunted, their leaves much deformed and their laminae greatly reduced (Pl. 3, fig. 4). Similar results are obtained with mixed infections of C.V. 1 with Hy.V. 3 or S.E.V.

The presence of C.V. 1 seems to have no effect on the concentration of S.E.V., Hy.V. 3 or P.V. *Y* in the sap of infected plants, for sap from plants infected with mixtures reacts with antiserum as strongly as sap from control plants infected separately with one of these viruses. Similarly, these viruses have no effect on the concentration of C.V. 1, for when inoculated to cucumber seedlings the dilution end-point of sap from plants with mixed infections does not differ from that of sap from plants infected with C.V. 1 alone.

### The stability of antisera to different viruses

Chester (1935) and Mushin (1942) found that antisera against unstable viruses such as P.V. *Y* and S.E.V. lost their ability to precipitate more rapidly than antisera against stable viruses such as tobacco mosaic virus. Chester suggested that this might be evidence for the view that antibodies are changed antigens and so would reflect the properties of the antigens that call forth their production. All our results are against such a view and indicate that there is no difference between the stability of antibodies to different viruses. It is true that if antisera are produced by the injection of equal quantities of sap from plants infected with P.V. *Y* and tobacco mosaic virus, the antiserum against P.V. *Y* may lose its ability to cause precipitation in a period of months whereas that of tobacco mosaic virus retains its specific activity for years. This difference, however, is only a reflexion of the quantitative differences between antibody content of the two sera and not of qualitative differences between the antibodies. Sap from plants infected with tobacco mosaic virus contains nearly 1000 times as much virus as sap from plants infected with P.V. *Y*, and when rabbits are injected with equal volumes of sap from the two kinds of plants the precipitin titres of tobacco mosaic virus antisera are much higher than those of P.V. *Y* antisera. If rabbits are given a course of injections with P.V. *Y*, or injected with concentrated virus preparations, antisera with higher precipitin titres can be made and these retain their precipitating power as long as do antisera to tobacco mosaic virus.

## Discussion

When we first described the ability of S.E.V. and Hy.V. 3 to protect plants against P.V. *Y*, and the apparent suppression of P.V. *Y* by S.E.V. and Hy.V. 3, we suggested that this might be used as evidence that these three viruses were fairly closely related, for they have many properties in common and no such phenomenon occurred when these viruses were mixed with potato virus *X* or tobacco mosaic virus, which have widely different properties. The further experiments described in the present paper, however, suggest different interpretations and that the phenomenon is quite different from the reciprocal protection, which is so successfully used to test whether viruses causing different symptoms are related strains.

Firstly, there is nothing in our knowledge of C.V. 1 to suggest that this is less closely related to S.E.V., Hy.V. 3 and P.V. *Y* than these are to one another; yet C.V. 1 is unaffected by the presence of these viruses and these are unaffected by C.V. 1. Secondly, although S.E.V. protects plants against P.V. *Y* and Hy.V. 3 and can replace them in tissues where they were previously established, M.E.V., which itself

protects plants against S.E.V., does not completely protect plants from P.V. *Y* or Hy.V. 3, and only partially replaces them. Thus, it is fairly clear that the interactions between S.E.V., P.V. *Y* and Hy.V. 3 are of a different type from those between related strains and it seems that the last two are unable to persist in cells infected with the first. This may throw some light on factors involved in the multiplication of viruses.

When leaves are rubbed with viruses, the number of local lesions obtained is little affected if the leaves are thoroughly washed soon after inoculation, and if such leaves are crushed within a few hours little or none of the inoculated virus can be recovered in the sap. This indicates that the viruses are rapidly adsorbed on or combined with some insoluble cell components and that they only occur free in the sap after they have multiplied. Definite evidence for the view that viruses occur in plants in combination with insoluble materials was obtained by Bawden & Pirie (1944). After expressing all the sap from infected leaves, they found that the solid residues still contained as much virus as that obtained from the sap, but that this was only liberated by special treatments. In view of these facts, the most reasonable interpretation of the experimental results obtained by infecting plants with mixtures of viruses and virus strains is that different viruses combine with and multiply at different specific sites, whereas related strains combine with the same sites. If a site in a susceptible cell is already occupied by one strain, then a second strain of that virus will be unable to attach itself and multiply. Thus, M.E.V., in spite of its low concentration in sap, could occupy all the sites suitable for the attachment of etch viruses and prevent the more virulent S.E.V. from becoming established.

M.E.V. does not prevent P.V. *Y* and Hy.V. 3 from becoming established, so that the ability of etch viruses to affect the concentration of these two viruses in sap is not due to blocking of multiplication-sites, but to interference with some later phase of multiplication. The simplest explanation is that the etch viruses affect the metabolism of cells so that some material, or enzyme system, which is normally present and essential for the multiplication of P.V. *Y* and Hy.V. 3, is no longer produced. The quantitative differences between the behaviour of M.E.V. and S.E.V. could then be explained on the grounds that the latter completely inhibits this essential constituent whereas the less virulent M.E.V. only reduces the quantity produced. This postulated material or enzyme system is presumably inessential for the multiplication of C.V. 1 or the etch viruses. It is likely that Hy.V. 3 also affects some other cell component that is concerned in the multiplication of P.V. *Y*, for this virus also interferes with the multiplication of P.V. *Y*. That there are many complex and interrelated factors concerned in virus multiplication is, of course, suggested by the apparent inability of viruses to multiply *in vitro*.

Previously the only type of interference described with plant viruses has been the reciprocal type and this has been restricted to virus strains serologically related. A similar type of reciprocal interference occurs with some animal viruses, e.g. influenza (Ziegler & Horsfall, 1944), which are not serologically related, so that the sharing of antigens is not essential for it. The nearest equivalent to the phenomenon of unilateral interference shown by S.E.V. and P.V. *Y* is probably the interference between two bacteriophages described by Delbruck & Luria (1942), who attribute the effect to the dominant bacteriophage blocking a 'key-enzyme' concerned in multiplication. Their results, however, are not strictly analogous to ours, for their dominant bacteriophage was unable to suppress the other if it was already fully established.

The disappearance of P.V. *Y* from the sap of leaves in which it was previously established in considerable quantities is a fact of some interest. A week or so after becoming invaded with S.E.V., sap from leaves which would have given a precipitation titre of 1/16 with antiserum to P.V. *Y* ceases to precipitate with this serum and no P.V. *Y* can be detected by inoculation to potatoes. It is possible that S.E.V. gives rise to conditions in the cells that actually inactivate P.V. *Y*, but these viruses have such similar properties *in vitro* that it is unlikely that C.V. 1 and the etch viruses could remain active in conditions that inactivate P.V. *Y* and Hy.V. 3. The rate of disappearance of P.V. *Y* from sap of cells infected with S.E.V. is approximately the same as the rate at which these viruses denature in expressed sap. It seems reasonable to assume that a similar denaturation to that *in vitro* is proceeding in the cell sap, and that the content of virus *Y* is kept reasonably constant only by the continued production of fresh virus. It is worth noting that a high virus content in expressed sap is obtained only with viruses that denature slowly *in vitro*. Thus, the great differences between the virus content of sap from plants infected with tobacco mosaic virus and P.V. *Y* may not indicate gross differences in multiplication, as usually believed, but may be due to the steady accumulation of one in the sap while the other is continually breaking down.

Fig. 1

Fig 2

Fig. 4

Fig. 3

Fig. 5

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## EXPLANATION OF PLATE 3

Fig. 1. Undersurface of a leaf from a President potato infected 6 weeks previously with severe etch virus, showing the distribution of veinal necroses.

Fig. 2. Enlargement of single leaflet from same plant as leaf in fig. 1.

Figs. 3–5. Photographs, to the same scale, of tobacco plants infected with cucumber mosaic virus (fig. 3), potato virus *Y* (fig. 5) and with both viruses (fig. 4). Note the much more severe disease produced by the simultaneous infection with both viruses, the plant is reduced in size and the leaves are greatly deformed.

(Photographs by V. Stansfield.)

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# The economic importance of the house sparrow, *Passer domesticus* L.: a review

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The present paper summarizes what is known about the economic importance and diet of the house sparrow. The species is widespread in the Old World, and in the last 100 years has more than doubled its original range through introductions, settling most abundantly in grain-producing areas of the world. Owing to a prolonged breeding season, its productivity is high and one pair will fledge about eight young per annum. The consequent very high density of the population in many areas is a major factor in making the sparrow a grain pest. Movements, migratory or local, are not marked in British sparrow populations.

A single sparrow eats about 6½ lb. of food per year, and as this is only partly and seasonally grain, it is the great numbers of the bird which constitute the economic menace. Cases are recorded in which 25% of an acre of wheat was destroyed by sparrows and in Russia up to 30% of a crop is generally lost. Table 1 gives a summary of the information available in the literature from stomach analyses.

*Grain.* Data are not very satisfactory for Britain, but in arable areas sparrows' food consists of 75–80% of grain. A lot of this is waste corn and poultry feed (three-quarters according to American figures), so that the limitation of such sources of food might form an important first step in controlling sparrows. In fruit-growing, garden and suburban districts far less grain is eaten (17%). Nestlings eat very little grain: in arable areas it may be as much as 40%, but in non-arable areas the diet is almost wholly animal. Data for other countries are more complete. Specimens examined from grain-growing areas (America, Turkestan) show that corn generally comprises half or more of the diet. In America the figure is 78%, but three-quarters of this is waste grain or feed. In Turkestan two

authors give about 50% and one 13%. In the latter case, owing to its great density, the sparrow was still a major crop pest. In park and suburban districts results are much like those from Britain: 30–40% grain is about an average figure. The same differences apply to nestlings: they may exceptionally take up to 50% grain but usually insects predominate, especially during the days just after hatching (50–78%).

*Weed seeds.* These are not eaten by nestlings. In Britain 10% in arable, 20% in non-arable areas are the figures given. The same percentage is given for America, but in Germany authors give 34%, and in Turkestan 46 and 83%. It is not certain that this part of the diet is entirely beneficial economically, since some of these seeds pass through the gut in a viable condition, so that the sparrow may be an agent for spreading weeds.

*Insect food.* The proportion of this item in the sparrow's diet varies greatly with locality and season. In non-arable areas 35% of the food may consist of insects, and this is taken mainly in the summer. In exceptional cases very large numbers of insects may be eaten. The food of nestlings consists mostly of insects in all localities, but in non-arable areas practically all the food is animal. Most of the species of insects taken are harmful to agriculture.

Damage to fruit buds, green vegetables and flowers may be of importance sporadically, but does not call for more than local action.

Apart from this, urban sparrows do no great damage and are useful scavengers. It is not thought that big flocks of sparrows in the harvest fields have moved out of the towns temporarily. In competition with other species of birds the sparrow probably menaces only the house martin (*Delichon urbica*) seriously by appropriating its nest. The evidence examined, though badly needing amplification, especially for Britain, suggests that house sparrows need controlling in arable, but not in other areas.

## Introduction

The economic position of the house sparrow (*Passer domesticus* L.) has been strongly debated during the last 100 years. Cases have been made out for its complete extermination and for its (almost) complete protection, and as yet no clear decision has been made as to the balance between its usefulness and destructiveness. This state of indecision causes needless waste of energy by people continuing to plead their cases and administrators trying to arrive at a satisfactory line to take. The present paper attempts to analyse the existing evidence in order to determine whether it constitutes an adequate basis for formulating a general policy towards the house sparrow, or whether further work is needed to settle the matter. The evidence will be considered under various headings of which the most important is obviously stomach-content analyses.

## The general biology of the house sparrow

### *Distribution*

In the Old World the house sparrow or a closely related form occupies the whole of Europe except Iceland and the most northerly part of Russia: in Asia it occupies all the western part except the middle of Arabia and north Russia: eastwards it reaches in the south to Ceylon and Burma, centrally it extends in a narrowing belt almost across to the east coast by the Amur River. In Africa *Passer domesticus* inhabits Algeria, Morocco and Tunisia, Cyrenaica and the Nile valley south to 12° north (Meise, 1940).

This is the limit of the sparrow's normal distribution. Other species of the genus *Passer* occur throughout the whole of the Palaearactic, Ethiopian and Oriental regions, their main centre being Africa.

The house sparrow has, however, enlarged its range in the most spectacular way by introductions. At the present date it has established itself in the following parts of the world: Africa: all Natal and southern Zululand (Roberts, 1940); Australia: all states except West Australia, extending north up the east coast as far as the tropic of Capricorn (W. B. Alexander, pers. comm.); New Zealand: both islands and many of the outlying islands (Oliver, 1930); North America: the whole of the United States, Mexico as far south as San Luis Potosi and Guadalajara (Heilfurth, 1931), Canada north to the limit of cultivation and along the railwords (Weaver, 1939); South America: practically all the Argentine Republic (W. B. Alexander, pers. comm.), all Uruguay, and isolated localities in Paraguay, Brazil (Wetmore, 1926) and Chile (Schneider, 1938). The Falkland Isles, Hawaii and Cuba have also been colonized: so has New Caledonia (Leach, 1928).

In all, the house sparrow's original range covered an area of roughly six million square miles. Extensions of this range in the last 100 years, whether by introductions or by unassisted spread, have amounted to slightly more than this figure. Thus in 100 years the sparrow has more than doubled its range and occupies about a quarter of the earth's surface.

### *Rate and characteristics of spread*

The best documented case is that of the U.S.A. The species was introduced in 1850, and by 1886 had occupied nearly a million square miles. In the subsequent year alone it occupied another half million square miles. By 1905 the whole of the States (three million square miles) were covered (data from Barrows, 1889; and Skinner, 1905). By now it has reached 600 miles down into Mexico (Heilfurth, 1931).

There appear to be two main causes for the facility

with which this remarkable spread has been conducted. First is the rapid expansion during the last 100 years of the world's grain-producing areas. A glance at any map showing the distribution of grain lands (e.g. map of wheatlands in Dudley Stamp, 1935) shows a remarkable conformity between these and the present range of the house sparrow. Furthermore, there is little doubt that since many of these new areas are engaged in exporting grain, traffic established for this purpose has greatly helped the movements of the sparrow. Mr W. B. Alexander gives the following interesting information: during the construction of the transcontinental railway in Australia a man was employed, upon his recommendation, to visit the camps at intervals as the railhead pushed out from occupied country and shoot the sparrows, which he presumed would follow this route to Western Australia. The sparrows were in fact doing this, but, thanks to the careful watch that was kept, they never reached Western Australia and the State is still free. In Siberia sparrows have spread northwards within the last 25 years, travelling up the Ob on grain boats and establishing themselves on the Yamal Peninsula (Grote, 1933).

The only large wheat-growing area in the world to which the sparrow has not yet penetrated is in Japan and China. In the former country Laburaki (1940), in a review of introduced animals, particularly mentions the absence of the sparrow. In view of the comparatively recent spread of the sparrow across the narrow farming belt of Asiatic Russia almost to the coast in the region of the Amur River, it will be remarkable if this area also is not occupied eventually.

The second factor, which contributes to the swiftness of the sparrow's spread, is its ability to colonize urban areas and live upon scraps and refuse, which even the most hygienically minded authorities cannot entirely abolish. This means that wherever the web of civilization spreads the house sparrow can follow and occupy an ecological niche which is not taken by local birds. Thus there is nearly always a thinly spread-out population, which can multiply rapidly whenever it comes into contact with farming areas. An interesting example of this is the way the species has spread in South Africa. Introduced at Durban about 1890, it has now colonized all Natal and the southern part of Zululand, living wherever there are buildings and settlements (Roberts, 1940). This is not a major grain-producing area and the sparrows are not particularly a nuisance, but if, for any reason, they were given a chance, they would no doubt multiply as they have in other places.

Another example of this kind of spread is found in the Kola peninsula. Sparrows reached Alexandrovsk in 1919 following the horses and their food brought by troops, and in 1923 they were also found to be established in Murmansk (Grote, 1933).

The eastward spread of the sparrow across the narrow belt of farming land in Asiatic Russia, mentioned above, may be attributed to both factors. Hartert (1910) put the eastern limit of the species as Irkutsk in the Lake Baikal region; when Hartert's book was brought up to date in 1932 most of the way to the Amur River mouth (Nikolaievsk) had been covered. Dresser (1902) considered that the sparrow followed the coaching road across Asia, but, since it is also well known that Russian agriculture was extending eastwards, this undoubtedly assisted the process. Boyd (1917) relates how the sparrow followed the army across the Sinai desert in the last war.

*Adaptability and resistance to environment*

There seems little doubt that the house sparrow can exist and thrive in any part of the world where man lives, with the possible exception of the tropics, providing that there is food. The limits of its northward distribution in America and Eurasia illustrate this strikingly. In Canada, Weaver (1939) shows that it is common up to the limit of cultivation and can spread farther north along the railroads, wherever there is food. Sparrows are known to survive at Churchill on Hudson's Bay, owing to their being fed with scraps during the winter. In Scandinavia and Russia they live up to 70° north wherever there are human settlements, and in Siberia they have followed the grain traffic areas northwards.

The adaptability of the house sparrow is nowhere better illustrated than in the big cities, where they find a living in the middle of modern traffic confusion. Another habit, which, no doubt, helps them to conserve energy and survive hard weather, is the building of winter nests. In this way they regularly pass the winter at a height of 10,000 ft. at Leadville, Colorado (Kalmbach, 1940).

*Productivity*

The breeding season of the house sparrow is protracted, when compared with that of most other British birds. All the same, it is difficult to get exact data on the distribution of breeding within this period and the number of broods raised on the average by each pair. Jourdain (in Witherby *et al.* 1938) states that the usual number of broods in Britain is 2–3: Kalmbach (1940) gives 3–4 as the figure for America. Probably a conservative estimate is nearer the truth, though this is formidable enough. Also local variations must be allowed for.

Clutch size also varies considerably. Jourdain gives 3–5 eggs as the commonest, while Kalmbach says 5–6. Weaver (1942) observed 38 nests at Cornell University, in which 180 eggs were laid (mean 4·7 per nest) and from which 127 young were reared successfully (mean 3·3 per nest).

Thus, supposing that half of the pairs had two broods and half three broods per season, 100 pairs of house sparrows would fledge successfully 825 young in a season. Allowing for the fact that these

fledgelings will be reduced rapidly, it is still obvious that they will make great inroads upon whatever they happen to be eating.

In Turkestan, where the bird is a migrant, conditions differ. Only one brood is raised, but clutches are larger: a count of the number of young in the nest gave a mean of 6·5 before 18 June and 3·86 after that date. Since by far the greater number of birds had bred in the earlier period, 100 pairs would still, under these circumstances, rear some 500 young (Arinkina & Kolesnikov, 1927).

*Density*

This obviously varies greatly with conditions and there are no reliable data about densities over large areas of country. However, a few actual figures for small areas will show the kind of congregations that occur. Russell (in Gurney *et al.* 1885) gives an instance where 150 sparrows were shot around one farm in a fortnight during the breeding season. This was in arable land. Dearborn (1912) records that during autumn 274 sparrows were trapped and 12 poisoned in one garden of a town of U.S.A. At another small town (4000 people) an average of 1000 nests were destroyed per season for 4 seasons. Buss (1942) records a case on a farm in Wisconsin, where the owner has shot sparrows continually for many years: between April 1941 and April 1942, over 700 were killed. However, the prize must certainly go to a village in Turkestan, where Kashkarov (1926) reports that over 1400 nests were counted in an area round the village of about 6 square miles.

*Changes in density.* Apart from the astronomical increases connected with the colonization of new territory, interesting information is available about decrease in numbers of the sparrow in certain areas. It seems quite clear from the data presented by Barrows (1889) that the density of sparrows at that time in some of the American towns was much greater than anything the British Isles have known. Many complaints are recorded about the large numbers roosting in creepers on houses and public buildings; these were so numerous that they fouled the creepers with droppings and killed them.

In the last 30–40 years, however, there has been a marked decrease in numbers. Eaton (1924) carried out counts of the number of sparrows seen per day from 1914 to 1922 and the mean figure fell from 13·7 to 5·6, while three endemic species counted as a check remained stationary. This decline in numbers is noted in many parts of the east United States, and many authors have attributed it to the disappearance of horses from the streets. Eaton points out that, although this may be an important factor, a decrease in numbers is also reported from localities, such as poultry farms, where this factor would not operate.

Decrease in the number of sparrows, especially in towns, is noted similarly in Canada (Weaver, 1939) and in France (Ménégaux, 1920).

*Movements*

For the most part, the house sparrow is extremely sedentary. Dearborn (1912) quotes a case where the population of a garden in America was exterminated by traps and poison, and the locality remained clear for 3 months in spite of the presence of many birds in the neighbourhood.

The most noticeable movements in this country and in America (Kalmbach, 1940) are the flocking parties that shift from the outskirts of towns and villages into the fields at harvest time. These consist largely of young birds at first, and they are later joined by adults as the breeding season comes to an end (Kalmbach, 1940). See also p. 65 below.

Ticehurst (in Witherby *et al.* 1938) mentions small passage movements noted along the east coast of England and across to the Continent, but these are of little importance.

In some parts of its range, however, the house sparrow is migratory. It is a summer resident only in Turkestan (Kashkarov, 1926).

*Amount of food eaten by a sparrow per day*

Upon this point very clear evidence has been assembled by Kalmbach (1940). An adult house sparrow weighs just over 30 g., and from records kept on captive birds it has been demonstrated that the normal daily ration is about one-quarter the weight of the bird. Thus the yearly consumption will be about 3 kg. or 6½ lb. Similarly, a nestling weighs about 20 g. when half-grown, and will eat about half its own weight per day. Thus in the 10 days of its life in the nest it will need about 100 g. or nearly ¼ lb. of food. Supposing that one is dealing with a population such as Kashkarov (1926) describes in Turkestan: about 100,000 birds will be present during the months of July and August on an area of about 6 sq. miles, and, supposing that only half their food is grain, they will consume during this period some 20 tons.

*Examples of damage by sparrows*

Few really satisfactory measurements have been made of the actual amount of damage done by sparrows to standing corn. Gurney *et al.* (1885) quotes a case in which a farmer thrashed separately 2 acres of his crop. The difference in yield between the first acre, which came from an untouched part of the field, and the second area, where sparrows had been busy feeding, was 16 bushels, or just over a quarter of a ton. Since a normal yield per acre of wheat in this country is about a ton, the sparrows had cleared over 25 % of the particular acre investigated. This represents the amount needed to feed about 700 sparrows for 2 months, supposing they were eating nothing but grain. Clearly, however, the acre chosen would be the one that had suffered the most spectacular damage, and no index is given of

the general amount of damage suffered by the crop. Kashkarov (1926) estimates that about 30% of the wheat grown at Tashkent, Turkestan, is destroyed by sparrows, but the figure is not so great farther north. In some places barley, millet and oats are not sown in the spring on account of the sparrow plague. The variation from place to place in the amount of damage done is illustrated by the detailed observations of Arinkina & Kolesnikov (1927), who marked off quadrats in numbers of wheat fields and calculated the percentage of the crops damaged from these samples. At Stalino the mean loss per cent was 30 (extremes 0·1 and 74%); at Pskent the mean was 13% (extremes 2 and 30%). This damage is all said to be done quite early, while the corn is still soft.

These figures, though not directly applicable to conditions in Great Britain, are nevertheless extremely interesting, since they give an idea of the order of loss that may occur.

## The food of the house sparrow

The data in existence upon this subject are sufficient to give a fairly good idea of the sparrow's economic status. A tabular summary has been prepared (Table 1), so that the results of each investigation can be compared more easily.

### *Proportion of cereal crops in the diet*

*Great Britain.* Three investigations have been made in this country and the results agree fairly well. In each case where birds were examined from an arable district corn predominated largely in the diet (75–81%). Gurney *et al.* (1885), who gave a seasonal summary of the relative abundance of each food item, and Florence (1912–15), whose data have been analysed by the writer, both agree that corn appears in the stomach contents most frequently in all seasons.

However, as Ritchie (1931) has pointed out, a large amount of this, especially in the winter months, must be waste grain, lying about the stack yards and granaries. It is probable also that a further quantity comes from chicken runs. This has been demonstrated well in the U.S.A. by Kalmbach (1940), who makes a distinction in his analysis between 'feed' and the various grains. These figures are discussed below, but it is important to note that the amount of grain eaten never rose above 39% stomach contents in any one month, while the average for the year was only 17%. 'Feed', on the other hand, formed the most important item of the diet in every month, reaching 84% in February, and averaging 60% for the year.

If we suppose that these conclusions apply to Great Britain, then it is clear that here is one way of approaching the sparrow problem—mainly by cutting out the supply of waste grain, which forms the greater part of the diet. In fact, an experiment on these lines is probably being performed for us at the moment. To the gradual reduction of the waste corn by elimination of the horse from agriculture, war-time conditions have suddenly added a vast reduction in poultry. *A priori*, one might expect one or more of three things to happen: that the sparrow would eat more grain from the fields and ricks; or, if this is not available (e.g. in the winter) that it should change its diet and eat more insects and weed seeds; or, lastly, that it should decrease in numbers.

In any case, in view of Kalmbach's results, it seems likely that the elimination of broadcast feeding of chickens, and the enclosure of runs with netting of finer mesh, would be a profitable start in tackling the sparrow problem.

### *Local variation*

Figures given by Collinge (1912, 1914, 1924–7) make interesting comparisons between the composition of the diet in different districts. It is important to bring out the variation because it not only means that the house sparrow is adaptable as regards food, but that its economic status may vary with locality. From a number of birds collected in a fruit-growing district Collinge showed that cereals composed only 17% of the food, but injurious insects 35% and weed seeds 20%. It has been shown by other workers that house sparrows in the breeding season tend to eat more animal food, such as they give to their young, and Collinge's figures may apply to this season (there is no explicit statement about this). Nevertheless, the difference between fruit-growing and arable areas is amply demonstrated.

### *Food of nestlings*

Like most young birds, nestling sparrows need a high percentage of protein in their diet, and this is the main peg upon which the defence of the sparrow is hung. Russell (in Gurney *et al.* 1885) gives a number of incomplete details of analyses, which he made to show that soft corn is taken a great deal for the young, but it seems probable that this could only be the case in late broods in this country and not at all in Scotland (Ritchie, 1931) where the corn ripens later.

Gurney himself estimated that the diet of young sparrows contained only 40% corn, the rest being mostly caterpillars and beetles. These figures are presumably from arable areas. Collinge (1912, 1914, 1924–7) examined 200 nestlings from a fruit-growing area and found that their food was almost entirely animal: 122 from a suburban area had also been fed exclusively on invertebrates, apart from kitchen refuse. The summary for the total number examined (487) gives a result of 95·5% animal food. Unfortunately, Collinge does not explain where the balance of the specimens came from, but it seems unlikely that they were from arable areas, in view of other people's results. Therefore, for Britain we have to

TABLE 1. *Summary of stomach content examinations of the house sparrow*

| Name | Date | No. of specimens examined | Method | *Food in Spring | Summer | Autumn | Winter | Proportions for year % | Nestlings % | Conditions |
|---|---|---|---|---|---|---|---|---|---|---|
| Gurney *et al.* | 1885 | 694 | Occurrence % | Corn<br>Greens<br>Seeds<br>Seed corn<br>Insects | Corn<br>Greens<br>Seeds<br>Insects | Corn<br>Seeds | Corn<br>Seeds | Corn 75<br>Seeds 10<br>nsects 4 | Corn 40<br>Insects 50<br>(Lepid. larvae 40,<br>Coleoptera 10) | England<br>Arable |
| Collinge | 1912–27 | 758 | Volumetric | — | — | — | — | Cereals 75<br>Seeds 10<br>Insects 5 | — | England<br>Arable |
| | | | Do. | — | — | — | — | Insects 35<br>Seeds 20<br>Cereals 17<br>Buds 9 | — | England<br>Fruit-growing district |
| | | 476 | Do. | — | — | — | — | — | Inj. insects 88<br>Mix. veg. 4·5<br>(total animal 95·5) | England: 200 from fruit-growing district, 122 from suburban district |
| Florence | 1912–15 | 146 | Occurrence % | Corn<br>Insects | Corn<br>Weeds<br>Insects | Corn<br>Weeds | Corn<br>Weeds | Corn 81<br>Weeds 15·5<br>Insects 3·5 | — | Scotland N.E.<br>Arable |
| Barrows | 1889 | 522 | Do. | — | Cereals 42 %<br>Veg. 20 %<br>Weeds 19 %<br>Insects 13 %<br>Fruit 6 % | — | — | — | | U.S.A., urban and park area, Washington, D.C. |
| Kalmbach | 1940 | 4848 | Volumetric | Feed<br>Cereals<br>Insects<br>Weeds | Feed<br>Cereals<br>Weeds<br>Insects | Feed<br>Weeds<br>Cereals | Feed<br>Weeds<br>Cereals | Feed 60<br>Cereals 18<br>Weeds 17<br>Insects 4 | — | U.S.A. All parts |
| | | 2819 | Do. | — | — | — | — | — | Insects 68<br>Feed 30 | Do. |
| | | | | | | | | May–August | | |
| Arinkina & Kolesnikov | 1927 | 990 | Occurrence % | — | — | — | — | Corn 50<br>Weeds 46<br>Insects 4 | — | U.S.S.R.<br>Turkestan |
| | 1927 | 1231 | Do. | — | — | — | | — | Insects 70<br>Corn 19 | Do. |
| | | | | | | | | May–August | | |
| Rusinova | 1926 | 1323 | Do. | Weeds | Weeds<br>Corn | — | — | Weeds 83<br>Corn 12·5<br>Insects 3 | Insects 77·8 | Do. |
| Schleh | 1883–4 | 263 | Do. | Insects<br>Weeds<br>Corn | Corn<br>Weeds<br>Insects | Corn<br>Weeds<br>Insects | — | Insects 35<br>Weeds 34<br>Corn 31 | Insects 50<br>Corn 50 | Germany. Garden on edge of town |
| Musson | 1904–5 | 109 | Do. | — | — | — | — | Cereal 88<br>Weeds 47<br>Insects<br>(harmful) 15·5<br>(neutral) 31 | — | New South Wales. Grain area |

* Items are given in order of importance.

rely upon Gurney's 50-year-old records for corn-growing land.

*Data from other countries*

*Europe.* For other European countries we have only the work of Schleh (1883–4) in Germany, which was done as long ago as that of Gurney; however, he gives his data in their entirety, and not merely in percentages, as so many authors do. The locality, where his work was done, was hardly true arable country, since it was a garden on the edge of a town: however, there were fields away to one side.

His estimate for the whole year is somewhat intermediate between the figures of Gurney for an arable area and of Collinge for a fruit-growing area. Corn, insects and weed seeds all occur about 30% times. A closer analysis of Schleh's data shows that this figure of 30% for insects is made up mainly during the breeding season (see Table 2).

TABLE 2. *Variation in the composition of the house sparrow's food during the year (data from Schleh)*

| | Insect | | Corn | | Weeds | |
|---|---|---|---|---|---|---|
| | Occurences | % | Occurences | % | Occurences | % |
| May–July | 52 | 47 | 16 | 15 | 41 | 38 |
| Aug.–Oct. | 9 | 17 | 26 | 49 | 18 | 34 |
| Nov.–Mar. | 4 | 18 | 14 | 64 | 4 | 18 |

Thus in summer and winter, when in an area which is not wholly arable, corn forms 50% or more of the sparrow's food.

As far as nestlings are concerned Schleh is careful to point out that generalized statements about their diet must not be accepted too much at their face value, because of the very close correspondence between the nature of the food and the locality. However, even in his specimens, which came from the same locality as the adults and from various German towns, 50% of the food was corn. This, no doubt, may have been largely wasted grain, but the results prove that young sparrows may be reared on a diet half of which is vegetable. Therefore, it looks as if the great preponderance of animal food noted by, e.g. Collinge, was probably due to greater availability during the nesting season. This does not invalidate the suggestion that nestlings need a high protein diet, but merely emphasizes that not more than half need be animal.

*U.S.A.* American work on the food of the sparrow needs to be treated in some detail because it is extremely thorough, and has been based on more specimens than all the rest of the work put together.

By far the most important work is that of Kalmbach (1940) who is the only author to make a distinction between waste grain and chicken feed, and grain from the corn fields. His results are surprising, for they show that by far the most important item in the diet during any month is 'feed'. The amount of this taken varies from 31% in September to 84% in February, while for the year the average is 60%. Compared with this the amount of grain taken is small: there is a peak during the harvest, when the figure reaches 40%, but the average for the year is only 18%.

This analysis shows clearly the extent to which the sparrow lives on the 'crumbs from our tables'. Cutting out this source of supply would certainly have a depressing effect upon the sparrow population and is the first obvious step that could be taken.

The exhaustive work of Kalmbach has rendered out of date the earlier report made by Barrows (1889) at a time when the house sparrow was at the highest point in its rate of spread over the United States. The results of his dissections are interesting, however, because most of the material came from a suburban area (Washington, D.C.) and the non-arable/arable contrast is shown as clearly as in the British results. Barrows found only 42% occurrences of cereals (presumably mostly 'feed'), other vegetable matter and fruit 26%, insects 13% and weed seeds 19%.

Considerable attention was paid by Kalmbach to the food of nestlings. Generally speaking, the larger proportion of animal food, noted above, is confirmed in his results. Grain, mostly 'feed', composes on the average 32% only of the diet, the rest being invertebrates, predominantly insects.

These proportions are not constant throughout the nestling period. Kalmbach analyses his results according to the age of the young birds examined, and demonstrates that there is a progressive increase in the amount of vegetable food (practically all 'feed') eaten. During the first 3 days from hatching this composes only 9%, but during days 4–6 and 7–fledging the corresponding figures are 33 and 48%; there is a related decrease in the percentage of insect food taken. So by the time it is fledged the young sparrow is sufficiently accustomed to vegetable food to go practically straight on to an adult diet. This point is also confirmed in the data given by Schleh, in which he distinguishes between adult and young birds during the summer. There is no significant difference between their diets.

Kalmbach also confirms Schleh's contention that the diet of nestling sparrows varies greatly with the locality. He gives separate figures for four localities, three of which were rural. The urban sample shows the highest proportion of vegetable food (43%), while of the three rural areas two are about average (33–34%), the other remarkably low (7%): at this last locality the young had been fed almost entirely upon grasshoppers.

*U.S.S.R.* The rather peculiar conditions in Turkestan have been mentioned previously. In the first place the sparrow here (*P. domesticus indicus*) is migratory; secondly, it nests for a great part out in

the fields themselves, and lastly, its density is remarkably high. Two other species, *P. hispaniolensis transcaspicus*, another migrant, and *P. montanus dilutus*, a resident, are also pests in the grain fields here. Two reports have been published concerning different areas in the neighbourhood round Tashkent. The results of Arinkina & Kolesnikov (1927) are not so greatly different from European and American ones. Grain (in this case mostly, if not all, direct from the fields) composes about half of the food taken, the next most important item being weeds (46 %) and finally a small proportion of insects (4 %). However, the data given by Rusinova (1926), which are extensive and complete, show very different tastes: by far the greater part of the food here is weed seeds (84 % of occurrences), while grain of all kinds is only 13 %. Figures for all three species together show that the highest percentage of corn found (23 %) was in mid-June, while a smaller peak occurred in August.

It is obvious that the damage done to crops in this neighbourhood arises not so much from grain being highly favoured in the diet, but from it being later eaten direct from the fields (in contrast to the 'feed' taken by American birds) and from the very high density of the sparrows. If crop damage is a major economic problem in these circumstances, then it certainly must be much worse under the conditions described by the other Russian authors.

In these localities the food of nestlings presents an even greater difference from that of the adults than in other countries. Arinkina & Kolesnikov found that 70 % of the food was insects, and 19 % corn, and Rusinova reported somewhat more animal food (78 %).

*Weed seeds*

These consist mostly of the common weeds of arable ground, species of *Chenopodium*, *Polygonum*, *Stellaria*, *Rumex*, *Ranunculus* etc. and seem to occupy rather different statuses in the sparrow's diet in different parts of the world. In Britain not much is eaten; fruit growing districts have the highest proportion (20 %). Arable areas in England have 10 %; in Scotland 15·5 %. American data show a similar state of affairs: Barrow's early investigation on birds mostly from Washington (park areas) revealed a proportion of 19 % weed seeds, while Kalmbach from his much more extensive material found 17 %. Schleh's figure is higher than these (34 %), and so is that of Musson for New South Wales (47 %).

To some extent, therefore, the house sparrow must have credit for destroying weed seeds. Certain species of weeds, however, are known to pass through the alimentary tract of the sparrow without being digested. Collinge (1924–7) grew 133 plants of 7 species from 54 droppings. This gives us no indication of the proportion of seeds eaten which are passed out in a viable state, but an average of 2–3 per faecal pellet means that a good number of weeds will be scattered about over quite large areas. However, more data are needed about this matter.

The most surprising data, as far as weed seeds go, come from Russia. Arinkina & Kolesnikov found that weeds composed 46 % of the diet, while Rusinova records the remarkably high figure of 83 %. The much lower figures in Britain may be due to cleaner farming and more intensive cultivation.

*Animal food taken by the house sparrow*

Most of the authorities give fairly extensive figures upon this subject, but the total amount taken, except under certain circumstances, is negligible. All figures given for the proportion of insects in the food are under 6 %, except in the following cases.

(i) Non-arable ground or ground with only a small proportion of arable. Collinge showed that in fruit-growing and suburban districts insects formed the most important part of the diet, but, unfortunately, he does not make it clear how many of his specimens were taken out of the breeding season. He gives 35 % as a general figure for the proportion of insects taken by adult sparrows in a fruit-growing area.

Barrows found 13 % insects in the specimens from Washington, and Schleh found about 18 % during the non-breeding season.

(ii) During the breeding season. House sparrows feed their small young almost entirely upon insects, and this seasonal change in habits affects their own diet to varying degrees. The analysis of Schleh's data illustrates this tendency well for Germany. During the nesting season adult sparrows' food is about half insects. Kalmbach's analyses by months show that in a general way insects compose about a tenth of the food eaten during May and June. He notes, however, particular cases where this average figure is much exceeded. In Salt Lake Valley, where a special investigation was carried out, large numbers of the introduced alfalfa weevil (*Hypera postica*) were being eaten (up to 30 %); in a lumber yard in Alabama great numbers of beetles (Scolytidae) were being taken. Thus it is clear that in certain circumstances the house sparrow may be economically beneficial.

Other authorities, however, indicate no, or little, effect on the adults' diet of the food being supplied to the young. Gurney *et al.* found that grain was still the predominant food, even when young were being fed. Florence noted no change in summer from the usual diet of corn. The Russian authors all agree that insects form only a very small part of the adults' food, though they may be bringing nothing else to their nestlings.

(iii) Food of nestlings. There is almost universal agreement among authorities that young sparrows at least are fed mainly on insects. These 10 days seem to be the only time during the sparrow's life when it is definitely an economic asset (see Table 1).

*Economic importance of the insects eaten*

Table 3 is a summary of the nestling's diet from the point of view of its economic importance (figures are percentages).

TABLE 3. *Economic importance of food of nesting sparrows*

| | Noxious insects | Neutral insects | Beneficial insects | Type of country |
|---|---|---|---|---|
| Collinge | 89 | 9 | 2 | Fruit-growing area |
| Arinkina & Kolesnikov | 31 | 68 | 1 | Grain area |
| Kalmbach | 59 | 12 | 28 | Unspecified |
| Schleh | 48 | 39 | 13 | Gardens |

The amount of insects of indifferent economic importance varies considerably. In all places examined, except Turkestan, there is little doubt that by far the greatest number of insects eaten are species harmful to agriculture. This is very strikingly shown in some of the instances, of which details are given. Thus in Kalmbach's colonies, which were living on alfalfa weevils, he calculated that individual broods were destroying nearly 2000 larvae per day: similarly in a number of young examined from Onaga, Kansas, 84% of the food was formed of grasshoppers.

Since the food of adult sparrows contains such a small percentage of insects it seems hardly worth while to consider their economic importance.

*Summary of house sparrow's food*

The results of these discussions upon the food of the house sparrow may be summarized as follows:

(i) In arable areas the adult sparrow eats a large percentage of grain.

(ii) Most of this, except during harvest period, is waste grain or chicken food.

(iii) In some parts of the world weed seeds are largely eaten (40–80%), but even so, if the density is excessive, the sparrow may be a crop pest, destroying up to 30% of crops.

(iv) In addition, the sparrow may act partly as a disseminator of weed seeds (to what extent is not accurately known at present).

(v) In suburban, park and garden and fruit-growing areas the sparrow will still eat a certain amount of corn if available (30%), though most of this is probably waste. The amount of insects in the diet, largely species harmful economically, increases in these areas, and the sparrow does a fair proportion of good here.

(vi) Young sparrows in the nest are fed mostly upon insects, mainly harmful species, but have already half switched to a vegetable diet by the time they are fledged. In exceptional cases large numbers of insect pests may be taken by sparrows to feed their young, and the adults may eat numbers also.

*Other damage attributed to sparrows*

A certain amount of damage, which is only slightly reflected in stomach analyses, is done by sparrows in orchards, gardens, and vegetable-growing areas. On occasions young plants and flowers are decimated by them and fruit may be attacked. Some authorities record evidence from stomach contents of these feeding habits. Collinge (1924–7) found 30% buds in stomachs of adults from fruit-growing areas; Barrows (1889) found 6% fruit in those he examined from Washington. This type of damage should not be exaggerated, because individual grievance makes a louder clamour than a nation-wide loss, but this trouble can be locally important. If these were the only depredations of which the sparrow was accused (as is, in fact, true in towns), then the amount of harmful insects eaten would more than counterbalance them.

*The urban sparrow*

In towns the house sparrow would seem on balance to be a useful scavenger, living mostly upon insects and kitchen refuse and committing only the mediocre crimes mentioned above. However, the charge is continually made that town sparrows move out into the country, when the corn is ripe, taking their families with them, and so much has been said upon this point that it must be discussed here.

Such data as we have show that the house sparrow is extremely sedentary. Of 53 birds marked and recovered in Oxford not one had moved more than 100 yd. or so. That there can be any great movement of sparrows from urban parts of towns seems unlikely in view of this evidence. Kashkarov (1926) states that the foraging range of *P. domesticus indicus* is up to 3·5 km., or about 2 miles. The figure may not be so great in Britain, but it does seem probable that birds living on the outskirts of cities and in the scattered small towns and villages may move into the fields during harvest time. The impression of numbers when a flock is seen together is so great that observers are tempted to think they are witnessing the effects of a migration. Such flocks, however, could easily be locally produced, since the percentage of young birds will be at its highest at this date. Dearborn's figure (1912) for a thousand nests destroyed yearly in a village of four thousand inhabitants shows this explanation to be the more reasonable. Further evidence is given by Hardy (1938), who found no diminution in a town sparrow population during harvest time.

*Relations with other species*

Many writers have sought to negative the amount of good which sparrows do in destroying insects, by asserting that their presence drives away other species of birds which would eat the insects anyway. In Britain there seems to be little evidence for this view,

except in the case of the house martin (*Delichon urbica*) which does not compete with the sparrow for food. To a certain extent the creation of new built-up areas, into which the sparrows can extend, is providing them with more ground, which other species would not use. For such species as would use them the presence of the sparrow in Britain at least seems to exercise no check. The blackbird and starling, for instance, have increased in such localities despite the presence of the sparrow.

As regards the house martin, there is little doubt that the house sparrow does compete with this species, to the latter's detriment. The competition here, however, is not for food but for nest sites. The martin's nest is an elaborate structure which takes a long time to build, and, if house sparrows appropriate it, when it is finished, as they frequently do, this obviously has serious consequences in depressing the martin's rate of increase. Since the martin feeds entirely upon insects caught in the air, a dense population cannot but be beneficial, and the sparrow is responsible for preventing the destruction of many insects.

An instance which illustrates this effect is given by Russell (in Gurney *et al.* 1885). At his own farm house martins declined until only two broods were reared in 1869; thenceforth sparrows were shot summer and winter, and by 1884 there were 170 martins' nests; by the middle of 1885 there were 237. A similar case from America is mentioned by Buss (1942) where the destruction of house sparrows caused an increase in the number of nests of cliff swallows (*Petrochelidon albifrons*), a bird similar to the martin, from a solitary one to 2000 in 38 years.

## Conclusions

The evidence is not sufficient to assess without doubt the economic position of the house sparrow in all habitats. Such an assessment will not be possible until a country-wide survey of the food of the sparrow has been made with proper attention to field observations.

From the present evidence (up to date for some other countries; out of date for Britain) it seems probable that the sparrow does harm in arable areas, and possibly also in other agricultural land and town premises, when these are adjacent to arable land and/or when the birds reach a certain density. Local action (e.g. trapping just after the breeding season), if carried out on the proper scale, should palliate the damage without wiping out the sparrow population. The latter ought not to be attempted until proper data have been gathered on food habits at the present day.

The present evidence suggests that the house sparrow may be useful in certain habitats (e.g. fruit-growing areas, towns), in all habitats at certain times of year (e.g. when the young are being fed), and in certain specific circumstances (e.g. insect plagues, etc.).

House sparrows probably do harm by competing for nest sites with house martins.

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# The influence of crucifers and mustard oil on the emergence of larvae of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber

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Hatching experiments were carried out with cysts of the potato-root eelworm. The effect of solutions of potato-root excretion mixed with root excretions of six types of crucifer seedlings was studied. With cress, white mustard, and black mustard, the emergence of larvae in the mixed excretions was very much less than that from control cysts in potato-root excretion alone. On subsequent return to potato-root excretion alone, larval emergence was found to be unimpaired in the case of white and black mustards; in the case of cress, however, the total emergence differed significantly from control. Similar effects, of both types, were obtained with certain dilutions of allyl isothiocyanate, the mustard oil of black mustard seed, in potato-root excretion.

## INTRODUCTION

In 1925 Morgan noticed that very few eelworm cysts were formed on the roots of potatoes grown in pots of infected soil together with 'mustard' plants. This observation was confirmed by Triffitt (1929, 1930) who also studied the effect on the emergence of eelworm larvae of the combined action of potato and 'mustard' root excretions, obtaining some striking results. Neither of these workers state which type of 'mustard' they used;* O'Brien & Prentice (1930, 1931), however, used white mustard, *Brassica alba*, in a field trial and some small-scale laboratory experiments which gave inconclusive results. It seemed desirable to continue this work in order to discover, if possible (*a*) whether these effects could be produced by other crucifers, and (*b*) the nature of the agent involved.

* Dr D. O. Morgan has since informed me personally that white mustard was used in both cases.

## MATERIALS AND METHODS

Small-scale field trials in 1941 and 1942 with a number of different crucifers were disappointing. Preliminary hatching trials, however, gave interesting results. Accordingly, two series of tests were carried out in June and August 1943; this section deals with the first of these.

### A. *June experiments*

The following crucifers were tested: white mustard, black mustard, turnip, rape, cress, and Brussels sprouts; in addition, lettuce, a non-crucifer, was included. Seeds were grown in sand in seed pans. Potato-root excretion was obtained by standing the whole root system of a growing potato plant in water

for 2 hr.; the solution was then divided into eight portions. White mustard seedlings, washed to remove the sand, were allowed to stand in one portion for 2 hr; six other portions were similarly treated with the other seedlings, leaving one to serve as an untreated control. The eight solutions thus obtained, six containing potato-root excretions plus excretions of different crucifers, one potato-root excretion plus lettuce excretions, and one consisting of potato-root excretions alone, were then tested on eelworm cysts using a single-cyst technique (Ellenby, 1943); each cyst was examined in a single drop of solution in a ring of vaseline 'stamped' on the floor of a Petri dish. Forty cysts, separated by flotation, were used with each solution, the original solutions prepared at the beginning of the experiment being used throughout the test. Larvae were counted and removed at 1- or 2-day intervals until, by the twelfth day, emergence had reached a low level. All solutions were then removed and replaced by drops of the original sample of untreated potato-root excretion. The additional larvae which in some cases appeared were counted for a further 4 days.

Table 1. *June experiments. Mean values of log* $(x+1)$ *and* S.E., *where* $x$ *= total emergence of larvae, per cyst, in both phases of the experiment*

| Lettuce *Lactuca sativa* | Rape *Brassica Napus* | Turnip *Brassica Rapa* | Brussels sprouts *Brassica oleracea gemmifera* | Cress *Lepidium sativum* | White mustard *Brassica alba* | Black mustard *Brassica nigra* | Control |
|---|---|---|---|---|---|---|---|
| $0{\cdot}586 \pm 0{\cdot}112$ | $0{\cdot}740 \pm 0{\cdot}112$ | $0{\cdot}463 \pm 0{\cdot}112$ | $0{\cdot}619 \pm 0{\cdot}112$ | $0{\cdot}542 \pm 0{\cdot}112$ | $0{\cdot}454 \pm 0{\cdot}112$ | $0{\cdot}748 \pm 0{\cdot}112$ | $0{\cdot}526 \pm 0{\cdot}112$ |

*Results*

The variation in total number of larvae produced, per cyst, is considerable in all groups; while the majority of cysts produce between 0 and 50 larvae, occasional values up to 400 are encountered. Treatment of data of this type, giving a much skewed distribution, has already been discussed in relation to a previous investigation with cysts of the same origin (Ellenby, 1944); a logarithmic transformation is used, the values of $\log(x+1)$, where $x$ is the number of larvae per cyst, being used in the analyses, instead of $\log x$, in order to avoid difficulties with cysts producing no larvae. The results of an analysis of variance of total number of larvae, per cyst, is presented in Table 1. The mean square between groups is slightly less than that within groups, so that $F = 1{\cdot}1$, while the standard error of the difference between any two means is equal to $\pm 0{\cdot}16$.

The mixed-root excretions, then, apparently produce no significant permanent increase or decrease in the total number of emerging larvae, per cyst; a very different picture, however, is presented when the emergences in the two phases of the experiment are compared for each group. The totals for the first 12 days when mixed-root excretions were used, (*A*), and the four subsequent days when all groups were examined in potato-root excretion alone, (*B*), are presented in Table 2; in the third column, the former values have been expressed as a percentage of the total for both periods. While the values for Control, lettuce, rape, and turnip, are very similar, cress is somewhat lower, and white mustard and brown mustard considerably lower, the latter approaching zero to indicate almost complete suppression of emergence. Triffitt (1930) obtained a reduction in emergence with mixed potato and 'mustard' root excretion only when the former was weak; strong potato-root excretions mixed with 'mustard' root excretions gave an increased emergence. The potato-root excretions used in the present work were undoubtedly above the minimum level necessary for maximum response; as almost complete suppression of emergence was obtained in some cases, the results fail to confirm Triffitt on this point; doubtless this is due to the greater strength of the crucifer excretions used.

Table 2. *June experiments. Total emergence in mixed excretions, A, and potato-root excretion alone, B. Forty cysts in each group*

| | *A* | *B* | $\frac{A}{A+B}\%$ | Productive cysts |
|---|---|---|---|---|
| Lettuce | 640 | 59 | 91·6 | 23 |
| Rape | 1248 | 184 | 87 2 | 21 |
| Turnip | 423 | 85 | 83·3 | 19 |
| Brussels sprouts | 468 | 54 | 89·7 | 21 |
| Cress | 328 | 130 | 71·6 | 24 |
| White mustard | 130 | 234 | 35·7 | 23 |
| Black mustard | 102 | 1080 | 8·6 | 22 |
| Control | 528 | 114 | 82·2 | 16 |

B. *August experiments*

The techniques used were essentially the same as in June. Tests were, however, restricted to black mustard, white mustard, cress, and lettuce. Potato-root excretions were treated with crucifer seedlings as before save that the number of seedlings was regulated to one seedling for every 0·5 c.c. of potato-root excretion. In view of the striking effect of black mustard seedlings, tests were also carried out

with the mustard oil, allyl isothiocyanate, which is formed in the seeds of brown mustard by hydrolysis of the glucoside sinigrin by myrosinase; three dilutions were used, 1/2000 (*X*), 1/20,000 (*Y*), 1/1,000,000 (*Z*), by volume, made up from the B.D.H. product in the same sample of potato-root excretion as the parallel experiments. By using the vaseline very hot, it was found possible to 'stamp' over 50 rings in a single 11 cm. Petri dish; this greatly increased the ease with which a large number of cysts could be examined: 120 cysts were used in each of the seven experimental groups, and 80 in a series kept in potato-root excretions throughout. Fresh solutions were prepared at 5-day intervals for all series. Emergence had practically ceased by the fifteenth day. Solutions were then removed, and all cysts left for 24 hr. in water; potato-root excretion of the last batch was then substituted and larvae counted at daily intervals for a further 6 days. Forty cysts of each of treatments *Y* and *Z* were, however, treated somewhat differently: these continued throughout in their respective solutions, the change to potato-root excretion alone being made with the remaining 80 of each group.

*Results*

There was no emergence in solution *X*, the strongest of the mustard oil solutions. The results of an analysis of variance for the remaining groups, carried out as before with values of log $(x+1)$, are presented in Table 3. The mean square between groups is considerably greater than that within groups; with $F=2{\cdot}62$, $p=$ almost 0·01, showing highly significant effects of some treatments on total emergence. In fact the difference from Control in the case of cress is highly significant ($p=0{\cdot}01$) and is significant too in the case of solution *Y* ($p=$ almost 0·02); in the remaining cases the differences are not statistically significant showing no permanent effect on total emergence for these treatments.

The separate totals for the two phases of the experiment are presented in Table 4; the totals for the 15 days of emergence in mixed excretions in column A, and those for the subsequent 6 days in potato-root excretion alone in column B. The two values (i) and (ii) for both solutions *Y* and *Z* are for the 80 cysts which were transferred to potato-root excretion alone, and for the 40 cysts which continued in the mixed solutions, respectively. In column 3, the total emergence in the first phase is expressed as a percentage of the total emergence in both phases. The values for both black and white mustard are again very much lower than the Control showing considerable suppression of emergence; cress, too, shows some reduction. Unlike the June results, lettuce in this series shows some effect; further investigation of this point may be of interest. The lowest value, 18·6%, is given by solution *Y* (1/20,000), the most dilute solution showing little effect. Comparison of the two values, (i) and (ii), for the former also show that while 417 larvae emerged from the 80 cysts after they were transferred to potato-root excretion alone, only 87 emerged during the same period from the 40 cysts which continued in the mixed solution; the corresponding values for solution *Z* are 123 and 11, showing that even a solution of 1/1,000,000, is not without effect.

TABLE 3. *August experiments. Mean values of log* $(x+1)$ *and* S.E., *where* $x=$ *total emergence of larvae, per cyst, in both phases of the experiment*

| Lettuce | White mustard | Black mustard | Cress | *Y* | *Z* | Control |
|---|---|---|---|---|---|---|
| 0·471±0·0535 | 0·499±0·0535 | 0·615±0·0535 | 0·383±0·0535 | 0·419±0·0633 | 0·453±0·0633 | 0·621±0·0633 |

TABLE 4. *August experiments. Total emergence in mixed excretions, A, and potato-root excretion alone, B*

| | | *A* | *B* | $\frac{A}{A+B}$% | Total cysts | Productive cysts |
|---|---|---|---|---|---|---|
| Lettuce | | 613 | 337 | 64·5 | 120 | 64 |
| Cress | | 603 | 343 | 63·7 | 120 | 50 |
| Black mustard | | 441 | 1372 | 24·3 | 120 | 58 |
| White mustard | | 361 | 766 | 32·0 | 120 | 72 |
| Solution *X* | | 0 | 0 | — | 120 | 0 |
| Solution *Y* | (i) | 95 | 417 | 18·6 | 80 | 41 |
| | *(ii) | *66* | *21* | *75·9* | *40* | *11* |
| Solution *Z* | (i) | 413 | 123 | 77·1 | 80 | 38 |
| | *(ii) | *147* | *11* | *93·0* | *40* | *14* |
| Control | | 1056 | 92 | 92·0 | 80 | 51 |

Solutions *X*, *Y* and *Z*: allyl isothiocyanate in potato-root excretion, 1/2000, 1/20,000 and 1/1,000,000, respectively.

* In mixed solutions throughout.

## Discussion

The results show the following: (1) the emergence of eelworm larvae is very much less in the presence of potato-root excretion together with root excretions of seedlings of cress, black mustard, and white mustard, than in the presence of potato-root excretions alone; (2) with the mustards, there is, apparently, no permanent injury, larvae emerging normally when the cysts are returned to potato-root excretions alone; with cress, however, there appears to be some permanent effect, as significantly fewer larvae subsequently emerge; (3) similar effects are produced by certain dilutions of allyl isothiocyanate, the mustard oil of black mustard seed.

Allyl isothiocyanate is derived from the glucoside sinigrin, present in black mustard seeds; white mustard seeds, on the other hand, contain sinalbin, which on hydrolysis by myrosinase, yields the mustard oil *p*-hydroxybenzyl isothiocyanate; the former was selected for test because of the marked effect of black mustard seedlings. The work of Stahman *et al.* (1943) suggests, however, that although different crucifers may contain different glucosides in their seeds, their roots may yield the same mustard oil; turnip, cabbage, black mustard, and white mustard were all found to yield phenethyl isothiocyanate, while horse-radish alone of those tested, yielded, in addition to this oil, allyl isothiocyanate. Cress was not tested; but if its roots also yield phenethyl isothiocyanate, the permanent reduction in emergence brought about by cress excretions may be due to a difference in concentration rather than to a difference in substances. It must be emphasized, however, that though the similarity of the effects produced by the crucifers and certain dilutions of mustard oil is very suggestive, it does not *prove* that a substance of this type is concerned in the action of the former.

This is not the first occasion on which isothiocyanates have been found to be of use in combating potato eelworm. Smedley (1939) tested a number of different isothiocyanates and found that phenyl isothiocyanate was particularly effective; practically no larvae emerged from cysts which had been immersed in a 0·001 % solution for 3 days, and a field trial with this substance incorporated into the soils absorbed on to talc gave striking results. Although a number of isothiocyanates were tested in preliminary experiments, the three mustard oils mentioned above in relation to the crucifers were not; in fact, the relationship of this class of compounds to the crucifers is not even mentioned. The conclusion is, therefore, unavoidable that this worker is unaware of the work of Triffitt (1929, 1930) and O'Brien & Prentice (1930, 1931) with 'mustard' seedlings, and because of this, presumably, attention is directed towards finding a concentration which will kill the cyst contents. The present results with seedlings and allyl isothiocyanate, however, show that emergence can be almost eliminated without damage to the larvae, that the presence of some crucifer seedlings, for example, interferes, in some way with the response of the larvae to potato-root excretions. Whether the presence of the former masks the presence of the latter, whether there is a chemical reaction between the two excretions, or whether the crucifer excretions actually act on the larvae, is unknown. Clearly, however, the fact of this interference must modify the approach to the use of isothiocyanates as a control measure; it is possible that the application of certain isothiocyanates to the drill, rather than everywhere, may be sufficient to prevent the emergence of larvae without actually killing the cyst contents. The destruction of the cyst contents may be more desirable but less practicable in view of the difficulty of incorporating chemicals in the soil; if the larvae are prevented from emerging, little objection would be raised to their remaining alive—within the cyst.

I have to thank Prof. A. D. Hobson for the encouragement he gave me during the course of the work.

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# Tyroglyphid mites in stored products

## Methods for the study of population density

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(With 1 Text-figure)

The known methods for detecting the presence of tyroglyphids are described. For coarse materials, such as seeds, sieving is recommended. For flour, it is better to examine exposed surfaces, or to keep samples in glass vessels and examine for the tunnels made by mites against the glass. Methods of sampling to provide information about the density and extent of infestations are described. Samples must usually be treated in the laboratory. While methods which do not involve the separation of the mites from the material are referred to, such separation is usually necessary. Mites may be separated from most materials by the modified Berlese funnel method of Chernuishev and Petrova; means of overcoming certain disadvantages are suggested. However, vigorous sieving is recommended for coarse materials, and a flotation method, using dichlorethylene, for fine materials such as flour. It is usually more satisfactory to examine and count the mites than to estimate the numbers volumetrically or gravimetrically. They are counted in a dish containing oil, placed on squared paper under a low-power microscope. A modification of this method, for dealing with large numbers, is described. Methods of expressing population density are briefly discussed.

Tyroglyphid mites ('cheese mites', 'flour mites', etc.) infest most stored products, particularly grain, flour and other cereal products, cheese and dried fruits. In Britain, the commonest, and the most important economically, is *Tyroglyphus farinae* (L.).

Under favourable conditions, tyroglyphids multiply rapidly so that dense populations are commonly found. In badly infested grain, for example, *T. farinae* may reach a population density of 250,000 mites per 100 c.c. of grain, i.e. about 1 % of the gross volume.

To obtain information about the usual forms, densities, and concentration points of infestations, it is necessary to study them in some detail. The methods described below have been used in such investigations; many are also useful in routine inspection work.

First, methods used for the detection of mites in storage places and in stored materials are described. It will be seen that some of these methods are also useful in quantitative work.

### Detection of mites

Descriptions of methods used in detecting tyroglyphids are scattered among the publications already listed (Solomon, 1943), with much repetition. While it is useful to collect the information, as below, it is not worth while to collect the references to each point.

Since tyroglyphids develop most readily under moist conditions, they are most likely to be found in places where moisture has been taken up by the food due to leaks in roofing, or damp walls or flooring, or exposure to a humid atmosphere, or where unduly moist products have been introduced. The mites may be visible in large numbers on the products or on the floors or walls of storage places. On the floor surrounding infested products there is often a sprinkling of fine dust containing mites, or heaps of mites with scarcely any dust. *T. farinae* may often be detected by the strong so-called 'minty' smell associated with it, and certain other species are reported to have characteristic odours. Coarse materials such as grain and other seeds may be sieved, and the siftings examined for mites with a lens or microscope. Samples of flour may be pressed down flat with a spatula or similar smooth instrument; if mites are present, they push up small mounds from the smooth surface in the course of a few minutes or less. A similar but less convenient method, used for flour or fine groats, is to mould the samples into cones or pyramids, the shape of which is visibly altered after some hours, due to the movements of the mites. The way in which mites attack wheat, by making a hole at the end of the germ, is so characteristic that this may be taken as a sign that it is, or has been, infested. Since mites infesting bulks of grain or other seeds are sometimes concentrated below the surface, any thorough examination involves taking deep samples.

There are other methods, more suitable for use when very few mites are present. If flour samples are placed in tall glass vessels, and left for a fairly long period, such as a day or so, any mites in the material are likely to come in contact with the glass and make characteristic sinuous tunnels against it. It is usually stated that the vessel should be illuminated from one side. Another method is to leave small attractive baits of well-moistened, freshly sterilized bran or

other material in contact with the stored products; the baits are taken up after a day or two and examined for the presence of mites. When infested samples are placed in glass vessels, the mites tend to climb up the glass within an hour or so, particularly if the sample is agitated occasionally (I have found this method is more effective if moist filter-paper is placed under the lid of the vessel, but even then it is not very reliable).

Chernuishev (1938) and Petrova (1940) described methods based on the principle of the Berlese funnel. This is a somewhat slow means of detecting mites, and is unsuitable, as described, for dealing with immobile hypopi, or the resting stages. A simpler and more rapid method is desirable for detection purposes.

For all except very fine materials, I have found sieving to be the most useful method, even when samples may contain very few mites. It is convenient for dealing with large numbers of samples. Grain, seeds, etc., may be agitated in a sieve which retains everything except the dust and smallest particles; these may be examined under a lens or low-power microscope for mites of all stages, including eggs, and also for the droppings, which are easily distinguishable under the microscope. A suitable sieve for grain is a metal one of 20–25 meshes per inch. Well-established infestations of *T. farinae* are most easily detected by smelling the material.

For the detection of infestations in flour, I have found that the most useful method is to examine the surface of the small accumulations of flour usually present on the outsides of the bags or on the floor beneath them. If numbers of mites are present, these surfaces have a finely granulated appearance, due to the separation of the flour into small particles by the mites in their continual movements. If large numbers of mites are present, the light brown of their pigmentation may be clearly visible on these flour surfaces. The above remarks apply also to the dust which falls from bags of many other products. For the examination of internal samples of flour, I have found the most useful method for rapid work to be the inspection of portions pressed flat, as described above. If more time is available, samples are kept in glass specimen tubes and examined for tunnels against the glass. A third alternative is the flotation method described in a later section.

The sifting of flour through fine sieves is not very satisfactory. Bolting-silk, about 80 meshes per inch, retains the larger mites but allows the eggs, larvae and smallest nymphs to pass through with the flour. White flour may be passed through finer meshes, but Newstead & Morris (1920), who sieved infested flour through bolting-silks of various degrees of fineness, found that even a silk as fine as 200 meshes per inch allowed some eggs of *T. farinae* to pass, although it held back a considerable portion of the flour.

### Collection of samples

Once the presence of mites has been established, the next step is to take a series of samples to provide information about the extent or degree of the infestation. For some purposes, it may be sufficient to take a number of samples at regular intervals throughout the bulk, so as to find the average degree of infestation of the material. Some idea of this may be formed by examining the samples on the spot, by some of the methods described above, but to obtain quantitative data it is generally necessary to take samples to the laboratory for treatment.

In large masses, such as bulk grain, or large stacks of bagged material, mite infestations are usually restricted to the moister parts, such as those near damp flooring or walls, or the outermost parts which have taken up moisture from the atmosphere. An infestation sometimes extends only a few centimetres into the bulk, and seldom more than a few metres. Hence it is generally necessary to pay special attention to the parts which are infested, to locate the points where the population is densest, and to find how far mites extend from these points into the general bulk. To map out an infestation in this way, it is desirable to take several horizontal and vertical series of samples, distributed on the basis of a preliminary inspection.

In bulk grain, deep samples may be taken with a spear provided with a hollow sliding nose-piece. A small spear, designed in this Laboratory (Oxley & Henderson, 1944), has been used for this work and found specially convenient. Grain in bags may be sampled by inserting a length of stout glass tubing obliquely upwards to the desired depth into the side or bottom of a bag. The grain may be collected, as it trickles out through the tubing, in a specimen tube held at the lower end.

For the sampling of bagged flour, an instrument has been made from a metal bicycle pump, the distal end of the barrel being removed, leaving an open end, the edges of which are sharpened. This is pushed into the flour through a hole in the bag, or at the top. When the pump is withdrawn, and the piston pushed home, it thrusts out a coherent core of flour, the desired parts of which are passed directly into sample tubes.

Numbered specimen tubes (4 × 1 in.) with waxed corks or rubber stoppers are convenient for holding samples of flour, grain, etc. The size of the sample taken is largely a matter of convenience in collection and treatment. I have generally dealt with samples of 30–40 c.c. of flour or grain, containing anything from 2 or 3 mites up to 100,000 or so.

### Determination of population density in samples

#### *Methods not involving separation of mites from infested material*

Maurizio (1905) arrived at a rough index of popula-

tion density in jars of meal, by counting the numbers of mites visible in the tunnels they made against the glass.

Howe & Oxley (1944) described a method for estimating the pest population in grain, by measuring the carbon dioxide production. A 1% concentration of $CO_2$ was produced in 24 hr. at 25° C. by grain containing 0·15 g. of *Tyroglyphus* per pound; this is roughly equivalent to 8000 mites per 100 c.c. of grain. Owing to the $CO_2$ production of the grain itself, the method could not be used for *T. farinae* populations less dense than 2000 or 3000 mites per 100 c.c. of grain. Nor is it appropriate when it is necessary to separate tyroglyphids from other mites or insects, or to estimate several species or life history stages separately.

### *Methods of separating mites from infested material*

For more accurate work, the mites must be separated from the food material before their numbers are determined. They may be most conveniently separated from coarse materials, such as grain and other seeds, by sieving. The effectiveness of this means of removing the mites depends partly on adequate agitation and on not having too great a quantity of material in the sieve: the layer of material should not be much more than 1 cm. thick. With wheat, very vigorous agitation is required if many of the germs have been hollowed out, as the mites tend to remain in the cavities. This is most noticeable when numbers of the germs have been partly consumed; up to 15% of the mites may remain in such grain after 2 or 3 min. sieving. For many purposes, however, the convenience of the method outweighs this disadvantage.

The funnel method described by Chernuishev (1938) may be used for almost all types of materials. The sample is placed on a screen and illuminated and warmed from above by an electric bulb. Beneath the screen is a large black funnel into which the mites fall in avoiding the increasingly hot and dry conditions above. At the bottom of the funnel is a vessel of alcohol or formalin solution to catch the mites. Petrova (1940) described a similar apparatus, in which the electric bulb may be replaced by an electric heater, or hot sand, with a metal plate interposed to diffuse the heating. Chernuishev stated that all the mites were extracted from various seeds in 1–1½ hr. at 60° C. Petrova recovered 85–92% of the mites with which samples of flour had been infested, in 25 min. The method is somewhat time-consuming, although this objection might be overcome by having a number of replicates of the apparatus in use simultaneously. Another disadvantage is that the immobile stages (eggs, resting stages, immobile hypopi) cannot move from the material in spite of the physical stimuli. In dealing with the coarser products, this difficulty could be overcome by using a suitable sieve in which the material could be agitated, over the funnel, and then heating the residue in the sieve, as in the original method.

Special techniques have been devised for use with certain materials. Thomas & Horsfall (1939) described a method of separating tyroglyphids from manure by washing in water containing a wetting agent. The fluid was strained until a conveniently small volume, containing the mites, was obtained.

Two similar methods of separating mites from finely divided materials have been described. Smirnov (1938, 1939) treated flour with anti-formin, centrifuged the resulting fluid, and separated the centrifugate containing the mites. Shchastny (1939, 1940) treated flour with alcohol, centrifuged, stirred up with a saturated sodium chloride and glycerol mixture (1 : 1) and centrifuged again so that the mites and eggs came to the surface.

A simpler method is to separate the mites (sp.gr. about 1·0) from the flour (sp.gr. about 1·4) by flotation, without adding water, which would convert the flour to a paste. No doubt many liquids would be suitable. I have used dichlorethylene, $CH_2CCl_2$ (sp.gr. 1·25). The sample of flour is shaken up with this fluid in a long-necked flask, then more fluid is added to bring the level almost to the top of the neck. The mites and eggs rise rapidly to the surface, and are lifted out on small pieces of filter paper held in forceps. This method has been found very useful.

### *Counting or estimating the numbers of separated mites*

In determining the numbers of mites separated from a sample, it is often desirable to make separate estimates for different species, or different life-history stages. Where the species or stages are sufficiently different in size, it may be possible to separate the mites into the desired groups by treating them in a graded series of sieves, but generally it is more practicable to deal with them all together, making a separation into groups only by eye.

Henschel (1929) measured numbers of mites volumetrically, by dropping them into a microburette immersed in 70% alcohol. He counted the number of mites in a small volume in order to establish the relationship between volume and numbers. This method is convenient for large numbers when the average size of the mites in different samples is fairly uniform, or when only a rough estimate is required. It is very inaccurate if the average size of the mites varies much, as, for example, between predominantly young populations and predominantly adult ones. Moreover, it does not enable the separate estimation of different stages or different species.

The weighing of mites is open to similar objections, and to the further one that living tyroglyphid mites may take up or lose large amounts of moisture, according to the surrounding humidity, and vary correspondingly in weight (Schulze, 1924). Never-

theless, weighing is often useful as a rough method for dense populations, particularly when the population consists (almost) entirely of one species, and when the humidity does not vary greatly within a series of samples. For *T. farinae* populations, a typical relationship is about 280,000 mites per gram weight.

A more accurate method is to spread the mites out evenly in a Petri dish, for counting under a low-power binocular microscope. One means of spreading the mites out is to put water to a depth of about 0·5 cm. in the dish and drop the mites on to the surface film, where they remain for some time without sinking or escaping. They may be distributed over the film with a seeker, and counted with the aid of squared paper (0·5 cm. squares; white ink on black paper) fixed

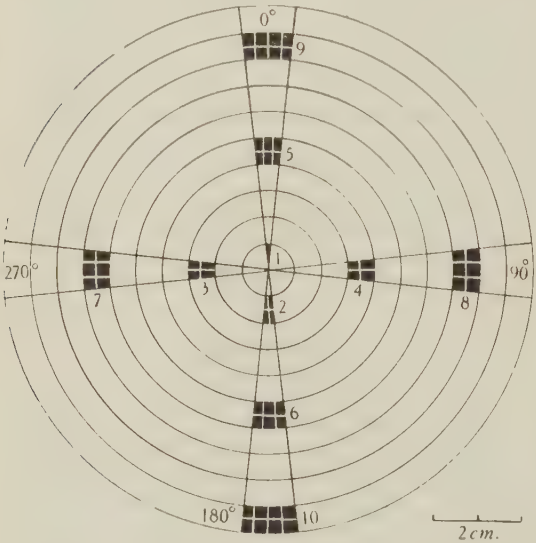


Fig. 1. Disk, marked with segmented sector, used in counting large numbers of mites.

under the bottom of the dish. It has been found more satisfactory, particularly if the mites have been taken from dichlorethylene, to use a heavy colourless oil such as medicinal paraffin or P31 Shell, instead of water. The mites sink to the bottom, and are thus closer to the squared paper, and are also less likely to drift about during counting. The dish is placed on a level bench under the microscope, with strong lighting from the side, and moved about gently and systematically until all the squares have been examined.

It is often impracticable to count all the mites in the dish. In such cases, the total number is estimated by counting the number over a segmented sector embracing 1/30 of the area of the dish. For this purpose, a card disk 12–15 cm. in diameter is prepared as follows (see Fig. 1): Four sectors of 12° are drawn from the centre in the positions 0, 90, 180, 270°, as shown in Fig. 1. Then a number of concentric circles of diameter $x$, $2x$, $3x$, ..., etc., are drawn, $x$ being about 0·6 cm. Each sector is thus cut into a series of segments. Then, proceeding from the centre outwards, the first segment is selected from the sector at 0°, the second from that at 180°, the third from 270°, the fourth from 90°, and so on. The selected segments are clearly marked, and subdivided into smaller areas to assist the counting. The disk is placed under the Petri dish, the dish being centred in relation to the concentric circles. The numbers of mites over those of the marked segments covered by the dish are counted, the total being then multiplied by 30 to give the number of mites in the dish.

The use of a sector corrects for the chief type of unevenness in the distribution of the mites—a thinning out near the edges of the dish. The distribution of the segments about the circle, as described, corrects to some extent for other types of unevenness. The card, once prepared, may be used with dishes of different diameters, convenient for counting various numbers of mites.

An idea of the degree of error to which a single estimation is liable is provided by the following data, from a test in which approximately 12,000 mites (*T. farinae*) were counted in a Petri dish of area 47 sq.cm. The count was done ten times, with a redistribution of the mites on each occasion. The results, after each had been multiplied $\times 30$, were: 12,900, 11,370, 12,330, 13,920, 12,690, 11,310, 10,140, 12,570, 11,940, 11,190. The maximum deviation from the arithmetic mean was $(12{,}036 - 10{,}140) = 1896$, or about 16 % of the mean. The standard deviation was 1074, or 8·9 % of the mean. For most purposes, this range of error is not too great.

### Methods of expressing population density

Pest population density has often been expressed as numbers per kg. or per 100 g. of food material. I have preferred to consider population density in relation to a fixed unit of space, by measuring the gross volume of each sample, and expressing population density as numbers of mites per 100 c.c.

As mentioned earlier, infestations tend to be localized, the greater part of the bulk being unaffected. It is therefore important, in discussing population densities, to state clearly whether one is speaking of (*a*) the density at particular sampling points, (*b*) the average density in the infested section, or (*c*) the average density based on a series of samples taken at random or at regular intervals throughout the whole bulk. At least one author (Chernuishev, 1939, discussing degrees of infestation of grain) has failed to make it clear which system was being used.

The investigation of this subject forms part of the programme of the Pest Infestation Laboratory, Slough, and the paper is published by permission of the Department of Scientific and Industrial Research.

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# The use of cobalt salts as indicators of humidity and moisture

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To meet the need for a simple method of measuring humidity in small spaces, a new method of using paper impregnated with cobaltous salts has been developed. Cobalt chloride paper is blue at low and pale red at high humidities, with a series of lilac colours between. There is a close correspondence between colour and relative humidity, although the colour is influenced slightly by temperature. It is shown that the colour is determined chiefly by the relative quantities of cobalt chloride and water in the paper. The paper is impregnated by dipping in a solution of $CoCl_2.6H_2O$. The time required for a piece of $CoCl_2$ paper to reach equilibrium with the atmosphere depends chiefly on humidity and temperature; up to 2 hr. exposure is allowed. Colour standards for the matching of test papers are prepared by exposing papers over constant humidity solutions and then sealing them up in liquid paraffin between opal and clear glass. It is possible to measure humidities from 40 to 70% R.H. to the nearest 2% R.H., and above this range to the nearest 5%, except for low humidities at low temperatures.

Various colloid substrates other than paper, including mercerized cotton, cause the blue colour to persist at somewhat higher humidities; possible uses of this are suggested. Certain salts, such as potassium thiocyanate and sodium thiosulphate, produce a similar, but much greater effect, and also produce stronger colours. The method can thus be adapted for accurate use at higher humidities. Alternatively, the addition of zinc chloride or certain other salts adapts the method for use at low humidities.

Papers more suitable for use at high humidities have been prepared with cobalt thiocyanate solution. A further improvement is the use of pure cotton tissue paper. Methods of impregnating the paper are described, also the above-mentioned method of preparing standards. Approximate corrections for temperature are given. The influence of temperature and humidity on the time taken to reach colour equilibrium is described. An exposure of 30 min. is sufficient except at very high humidities, where up to 2 hr. may be necessary. Corrections are given for use when exposures other than 30 min. are allowed. The standards cover humidities above 50% R.H. at intervals of 5% R.H.; test papers can be estimated to about the nearest 1% R.H. But under unfavourable conditions, errors may amount to as much as ±5% R.H., unless the corrections are used.

## 1. Introduction

Biologists and others who wish to keep a check on humidity conditions, in the course of experimental or observational work, often find that none of the established methods of humidity measurement is suited to their particular needs. It is difficult, e.g., to measure the air humidity in small culture jars and vials, in samples of stored products and other materials, and in cracks in floors, spaces between bags of grain, and other small spaces which provide micro-environments for various pests. In many such cases, a high degree of accuracy is not necessary, and what is required is a method which is simple to operate, does not require cumbersome apparatus, and will indicate the humidity in small spaces.

With these points in mind, the writer set out to determine whether cobalt chloride paper could be used for such purposes. In the course of the work, various developments of the method of using filter paper impregnated with cobalt chloride have been investigated, and many possible applications of these methods have become apparent.

## 2. Colours of paper impregnated with cobalt chloride

Dehydration of the ruby red crystalline $CoCl_2.6H_2O$, by exposure over a drying agent, gives rise to a material of lilac colour. When paper is strongly impregnated with cobalt chloride, there is an increased tendency to formation of the blue complex: thus the colour ranges from a series of blues at low humidities, through a series of blue-violets and violet-reds from about 50–70 % R.H., and at higher humidities through a series of light reds which are progressively paler up to 100 % R.H.

Table 1

| Paper ... ... | *a* | *b* | *c* | *d* | *e* |
|---|---|---|---|---|---|
| Strength of $CoCl_2$ solution | (Control untreated) | 0·26 *N* | 0·26 *N* | 0·26 *N* | 2·10 *N* |
| Volume of solution used, in ml. | | 0·11 | 0·33 | 0·99 | 0·369 |
| Calculated wt. anhydrous $CoCl_2$ used, in mg. | | 1·86 | 5·57 | 16·70 | 50·31 |

It is well known that filter paper impregnated with cobaltous chloride assumes a blue colour when comparatively dry, as at low humidities, but becomes pink when moist, as at high humidities. Such paper has been used by plant physiologists for the measurement of the transpiration rates of leaves; this is done by measuring the time taken by the paper, in contact with a leaf, to pass from one standard colour to another, and comparing this with the time taken when in contact with a standard evaporating surface (Livingston & Shreve, 1916; Henderson, 1936). The paper has also been used as an indicator of sweating (Darrow, 1943). Cobalt chloride in collodion painted on insects has been used as an indicator of cuticular transpiration (Eder, 1940).

Another application of biological interest is the use of cobalt chloride to determine the amount of 'bound' water in various colloids (Hatschek, 1936; Kretovitch & Uschakova, 1940; Weidinger & Pelser, 1940).

Cobaltous chloride in the solid state is normally a ruby-red crystalline hydrate, $CoCl_2.6H_2O$; lower hydrates are also known (Friend, 1920). It forms a red solution in water. The colour of a strong solution changes to violet and ultimately to blue as the temperature is raised, or in the presence of various salts or of hydrochloric acid. It also becomes blue when dissolved in various organic solvents.

Such colour changes in cobaltous salts, including the chloride and the thiocyanate, have been the subject of many physico-chemical investigations. The colours are associated with various complexes, e.g. of cobalt and water, or of cobalt and chlorine. The more important work on this subject is contained or reviewed in the papers by Hill & Howell (1924), Basset & Croucher (1930), Howell & Jackson (see References), and Csokan, Kiss & Richter (see *Chemical Abstracts*, 1938–41).

Howell & Jackson (1937*c*) found that when filter papers were impregnated with different amounts of cobalt chloride, and examined in the absence of moisture, the absorption spectra were essentially the same in all cases, differing only in intensity. In the presence of moisture, however, the writer has found that the colours of such a series of papers differ among themselves.

For this work, pieces of pure cotton tissue paper, without loading or size, were used. They were cut from a sample of 'Electrolytic "A" Tissue', kindly supplied by Brittain's Ltd. Each piece was approximately 5·30 sq.cm. in area, and weighed 216 mg. when dried for several days over phosphorus pentoxide. Various amounts of standardized cobalt chloride solutions* were spread evenly over the papers from a capillary pipette (see Table 1). Each paper lay during this treatment on a sheet of glass, on which it was left until dry. Table 2 shows the colours of the papers, as estimated by eye, when exposed to various atmospheric humidities at 20° C.

It is thus clear that the colour assumed by an impregnated paper at a given humidity is influenced by the amount of cobalt chloride present. In view of the fact that the colour of cobalt chloride in hydrochloric acid is said to be determined by the relative concentration of chlorine ions and water molecules

* Made up from 'Analar' $CoCl_2.6H_2O$ and standardized by titration as described in § 3 (*a*).

(at constant temperature; Howell & Jackson (1933, 1936*b*)), this ratio was determined for the impregnated papers. They were exposed to an atmosphere of approximately 40% R.H. for some hours, and then weighed. The observed and calculated data (Table 3) show that there was a progressive colour change from red to blue as the amount of cobalt chloride (or the number of chlorine atoms) increased in relation to the amount of water present.

paper may be due to the fact that the colour is more difficult to detect in the weaker papers: most probably a higher proportion of the material must be in the blue form in the weaker papers before this colour can be detected by eye.

It is not known whether the paper renders some of the water non-available to the cobalt chloride. Such an effect would be appreciable only in weakly impregnated papers. Little is known of the extent

TABLE 2

| Paper ... ... | *b* | *c* | *d* | *e* |
|---|---|---|---|---|
| 5–10% R.H. (desiccator with $CaCl_2$) | R(b) | Rb | (r)B | B |
| 15% R.H. (desiccator with $H_2SO_4$ sol.) | R | Rb | (r)B | B |
| 40% R.H., approx. (laboratory) | R | R | Rb | rB |
| 50% R.H., approx. (laboratory) | R | R | Rb | RB |
| 70% R.H. (desiccator with KOH sol.) | R | R | R | R(b) |

Colour symbols: R = red, B = blue, R(b) = red with trace of blue, Rb = red with obvious blue component, RB = red and blue about equal, rB = blue with obvious red component, (r)B = blue with trace of red.

TABLE 3

| A. Paper ... ... | *a* | *b* | *c* | *d* | *e* |
|---|---|---|---|---|---|
| B. Total wt., in mg. | 226 | 226·4 | 235·3 | 257 | 318·7 |
| C. Dry wt. of paper, in mg. | 216 | 216 | 216 | 216 | 216 |
| D. Wt. $CoCl_2$ (anhydrous), in mg. | — | 1·86 | 5·57 | 16·70 | 50·31 |
| E. Wt. water (B–C–D), in mg. | 10 | 8·54 | 13·73 | 24·30 | 52·39 |
| F. Wt. anhydrous $CoCl_2$: wt. water (D : E) | — | 1 : 4·60 | 1 : 2·46 | 1 : 1·45 | 1 : 1·04 |
| G. No. of chlorine atoms: no. of water molecules $\left(D:\frac{E\times 129{\cdot}85}{18{\cdot}0156\times 2}\right)$ | — | 1 : 16·58 | 1 : 8·88 | 1 : 5·24 | 1 : 3·75 |
| H. Colour of paper (symbols as in previous table) | — | R | R | Rb | rB |

TABLE 4

| A. Paper ... ... | *b* | *c* | *d* | *e* |
|---|---|---|---|---|
| B. Humidity at which blue just visible (R.H. %) | 5–10 | 27 | 60 | 80 (approx.) |
| C. Total wt. at hum. B, in mg. | 220·7 | 231·6 | 264·3 | 373·0 |
| D. Dry wt. of paper, in mg. | 216 | 216 | 216 | 216 |
| E. Wt. $CoCl_2$ (anhydrous), in mg. | 1·86 | 5·57 | 16·70 | 50·31 |
| F. Wt. water (C–D–E), in mg. | 2·84 | 10·03 | 31·60 | 106·69 |
| G. Wt. anhydrous $CoCl_2$: wt. water (E : F) | 1 : 1·53 | 1 : 1·80 | 1 : 1·89 | 1 : 2·12 |
| H. No. of chlorine atoms: no. of water molecules $\left(E:\frac{F\times 129{\cdot}85}{18{\cdot}0156\times 2}\right)$ | 1 : 5·50 | 1 : 6·48 | 1 : 6·82 | 1 : 7·64 |

The final point for investigation was to determine whether all the papers changed from red to blue at the same value of the $CoCl_2$ : water (or chlorine : water) ratio. The papers were exposed at a series of humidities at 20° C., until the humidity level at which the first visible trace of the blue component appeared had been determined for each paper. The weights of the papers at these humidities were found by weighing in closed vessels. The results are given in Table 4.

In view of the wide range of $CoCl_2$ and water content in these papers, the similarity of the $CoCl_2$ : water ratios is much more striking than the comparatively slight differences. The gradual decrease in the water : $CoCl_2$ ratio from the strongest to the weakest

to which the sorption of the cobalt salt by the paper influences the behaviour of the salt with respect to water, beyond the fact that the sorbed salt is considerably bluer and less red than the free salt at the same atmospheric humidity. Hence, it is perhaps not surprising that no obvious correspondence appears between the above ratios and the equivalent ones determined for cobalt chloride + hydrochloric acid + water solutions by Howell & Jackson (1933, 1936*b*), nor between the above ratios and those of cobalt chloride (or chlorine) to water in the hydrates and complex ions described in the literature.

It may be concluded, however, from the above experiments, that the colour of impregnated papers

is determined chiefly by the ratio of cobalt chloride (or chlorine) to water in the papers. The first visible blue colour appears when the ratio weight of anhydrous salt:weight of water, rises above about 1:2, or when the ratio numbers of chlorine atoms:numbers of water molecules, rises above about 1:7.

The thermal effect studied by Howell & Jackson (1936*b*) was not considered in the experiments since the temperature was constant. It causes relatively slight variations over the normal range of laboratory temperatures. Since the colour depends chiefly on the proportion of cobalt chloride to water, and the amount of the former is constant in standard papers, the colour is in effect chiefly determined by the moisture-content of the impregnated paper. This is itself determined by the atmospheric relative humidity. Hence, it is to be expected that the colour of the treated paper will change *pari passu* with the relative humidity.

### 3. Cobalt chloride paper

The development of the method described below depended upon whether there was in fact a regular correspondence between the colours assumed by cobalt chloride paper and the atmospheric humidity. Comparison of standardized cobalt chloride paper with colour charts established that there did exist a high degree of correlation between the colour and the relative humidity.

#### (*a*) *Impregnation of the paper*

In preparing papers for this work, much stronger solutions of cobalt chloride were used than those employed by Livingston & Shreve (1916) and by Henderson (1936), so as to strengthen the colours and facilitate matching. A 25% w/v solution of 'Analar' $CoCl_2.6H_2O$ was adopted as the standard.* The method adopted for preparing the papers was a simplification of that used by the authors mentioned. Dried filter paper was immersed for 1 min. in the standard solution (at 20° C.), then mopped under pressure between two changes of desiccator-dried no. 1 Whatman filter paper, the pressure being applied as evenly as possible with a squeegee. The paper was then allowed to dry slowly at room humidity.

#### (*b*) *Measurement of the colours*

There are many ways in which the colours of the paper could be measured. Since there is a gradient of intensity from the dense blue to the pale red end of the range, a photometric method could be adopted. The possibility of using a sensitive photo-electric cell was ruled out on the grounds of expense and complexity, which would tend to defeat the purposes of the method.

The course adopted was to match the colours of the paper against a suitably calibrated series of standard colours. Sets of coloured glass standards were kindly prepared by the Tintometer Co. for trial use in a comparator. This method was, however, abandoned because it involved the use of temperature corrections (see § 3 (*d*)) and because of difficulties in accurately matching the colours of the paper with those of the glass. When means of intensifying the colours of cobalt papers are used (§§ 5 and 6), the latter difficulty may not be serious. The same observations apply to the use of printed or painted colour standards.

The colour standards finally used were prepared from pieces of the impregnated paper, allowed to come into equilibrium over standard humidity solutions. The papers were suspended in stoppered bottles over potassium hydroxide solutions, adjusted to provide a series of constant humidities (Buxton & Mellanby, 1934), and kept at constant temperature, 21° C.

It was necessary to keep the colours constant, while making these standard papers accessible for matching with test papers. The method of enclosing the standard papers in flat glass tubes sealed with wax and shellac was tested, but found inconvenient and not very reliable. Finally, the difficulty was overcome by mounting the papers in liquid paraffin, between pieces of glass. The oil effectively isolated the papers from the effects of external humidity changes. The colours still varied a little in accordance with temperature variations. This, however, was considered to be an advantage, since the direct effects of temperature on the colours of the test papers could be disregarded, provided the test papers and the standards were at approximately the same temperature at the time of matching.

As a means of intensifying the colour of the paper, white opal glass was used on the under-side, the upper glass being colourless. Finally, the two pieces of glass, with the paper between, were pressed together by means of a weight and sealed at the edges with liquid glue or sodium silicate (an account of this procedure is given in § 6 (*e*)). Standards were made for humidities at intervals of 5% R.H. from 30 to 100% R.H.

These standards retained their original colours for 6–12 months. Sets which had been in use for 5 months appeared to be unaltered, but after 16 months considerable changes in colour had occurred and they could no longer be considered satisfactory.

#### (*c*) *Time required to reach equilibrium*

Many measurements were made of the time required for the papers to reach equilibrium when removed from one humidity to another. A series of papers was exposed in each case, so that single papers

* If stronger solutions are used, centres of crystallization tend to develop on the paper, appearing as small red spots. The normality of the 25% solution was checked by titration against silver nitrate (Follard's method); the results indicated a normality of 2·165 $N$ as against the theoretically expected value of 2·169 $N$.

could be removed at intervals and matched with the standards. It was found that papers became equilibrated more rapidly to a fall in humidity than to a rise, except at very high humidities. At high humidities, they equilibrated somewhat more rapidly to a rise in humidity than to a fall, but both processes were slower than the corresponding ones at lower humidities. Providing the change in humidity was not very small (e.g. not less than 10% R.H.), the amount of the change had little effect on the time required for equilibration. The greater part of the colour change took place rapidly, in a few minutes, the remaining change being much slower.

At 21 and 7° C., and humidities up to about 85% R.H., 30 min. was a sufficient time for complete equilibration, as far as could be estimated by eye. At humidities above about 85%, an exposure of 2 hr. was required. Apparently, at these high humidities, final equilibrium was not reached even in 2 hr., the changes being continuous. However, since the standards had been exposed for 2 hr., this period was sufficient to ensure a correct match; a much longer exposure at R.H. over 85% would introduce errors.

An examination of the rate of change in weight of the papers following a change in humidity was made by weighing them suspended over standard humidity solutions by means of a fine wire passing through the lid of the vessel to a torsion balance; 30 min. was usually an insufficient exposure time to allow of complete equilibration, even at low humidities, but the subsequent changes in weight were relatively slight.

Obviously the soundest procedure would be to expose all test papers for the same time as the standards, i.e. 2 hr. But where the saving of time is important, an exposure of 30 min. is sufficient, at humidities below 85% R.H., to eliminate visible equilibration errors.

### (*d*) *Effects of temperature*

The effect of temperature on the colour of the paper is twofold: a direct effect, as a result of which the cobalt salt becomes bluer at high temperatures than at low (Howell & Jackson, 1936*b*, 1937*b*), and an indirect effect, due to the influence of temperature on the amount of moisture held by the paper-salt complex at a given humidity.

The direct effect can be studied by comparing the colours assumed by the same piece of paper, immersed in oil, at different temperatures. A fall in temperature from 20 to 0° C. caused colour changes equivalent to 4–5% R.H., in papers equilibrated at various humidities. A rise in temperature from 20 to 30° C. caused colour changes equivalent to 3–5% R.H. These effects may be neglected, since they operate equally in the test papers and standards—provided the test papers and standards are at about the same temperature when matched together. But if coloured glass or printed or painted colours were used as standards, or with a photometric method, corrections for the direct effects of temperature would be necessary.

The indirect effect is observed when papers are exposed at much higher or lower temperatures than that at which the standards have been prepared. Test papers were exposed for 2 hr. to various humidities at 6 and 30° C., then mounted in oil, and compared at 20° C. with standards which had been prepared at 21° C. The papers exposed at 30° C. read correctly as far as could be estimated by eye. Those exposed to humidities of 75% and over, at 6° C., also read correctly, but papers exposed at 6° C. to 65% R.H. read 1·5% R.H. too high, to 50%, 2·5% too high, and to 40%, 3·5% too high.

There is thus an appreciable error due to the indirect effects of temperature when papers are exposed to medium and low humidities at temperatures much lower than that at which the standards have been prepared.

### (*e*) *Hysteresis effects*

The behaviour of any system with a colloid component is likely to be influenced by its previous treatment. Attempts were made to determine whether the colour assumed by the paper at a given humidity was influenced by the humidity to which it had been exposed previously. However, no such effects were detectable in papers matched against the standards by eye.

### (*f*) *Precautions against damage*

Papers which were kept for some time at humidities about 95–100% R.H. were found to be weaker in colour than normal papers. This appeared to be due to the leaching out of some of the cobalt salt, and no subsequent humidity exposure would correct it.

Wetting the paper washes off the cobalt chloride or at least makes it uneven and blotchy in appearance. It is, therefore, advisable to keep the paper at a fairly low humidity before use, and to guard against the formation of dew in the container following falls in temperature.

Many liquids and gases damage the paper by their effect on the cobalt chloride. It is necessary to avoid contact with acids and alkalies, or exposure to ammonia vapour, which discolours the paper. Removal of dust and small foreign bodies which have become attached to the test paper during exposure is best done after it is immersed in the oil.

### (*g*) *Accuracy of the method*

The accuracy attainable by this method depends on a number of variable factors. Matching tends to be more accurate in strong daylight than by most artificial lighting. There is a somewhat variable personal factor, owing to variations in the ability of individuals to distinguish small colour differences;

probably this improves with practice. The clearest colour gradient is over the humidity range 40–70% R.H., with the result that the method is at least twice as accurate over this range as at higher or lower humidities.

Since the personal factor necessarily enters into any test of accuracy involving matching the colours by eye, a formal test of accuracy could only be made by use of a photometric method. The experience of the writer is that it is possible, with care, to measure humidities from 40 to 70% R.H. to the nearest 2% R.H., and above or below this range to the nearest 5%. As may be seen from § 3 (*d*), this degree of accuracy is not possible for low humidities at temperatures much lower than the exposure-temperature of the standards, unless corrections are applied.

Some of the modifications discussed in the following sections enable more accurate measurements to be made.

a number of colloids with a 30% w/v solution of $CoCl_2.6H_2O$. After the excess moisture had evaporated, they were exposed to various humidities, and the colours examined. Particular attention was paid to the appearance of the lilac colour corresponding to that of solid cobalt chloride dried over calcium chloride, which appeared to be the same as the colour developed on the impregnated filter paper at about 55% R.H. Lilac colours intermediate between this and blue or red were listed as blue-lilac and lilac-red.

As shown in Table 5, the lilac colour developed by the paper at 55% R.H. appeared at about 65% R.H. when the salt was sorbed by various protein materials. While such materials are much less stable and reliable substrates than filter paper, these or other colloids might be used for special purposes, e.g. in cases where the appearance of the blue, red or lilac colours might serve as signals of humidity

TABLE 5

| Substrate | 30% R.H. | 55% R.H. | 60% R.H. | 70% R.H. | 80% R.H. |
|---|---|---|---|---|---|
| Filter paper; cellophane; muslin | Blue | *Lilac* | *Lilac*-red | Red | Red |
| Silk (bolting-silk) | Blue | Blue | *Lilac* | Red | Red |
| Filter paper impregnated with $CoCl_2$ solution containing 10% w/v albumin | Blue | Blue | Blue-*lilac* | *Lilac*-red | Red |
| Gluten; gelatin; dried egg albumin | Blue | Blue | Blue-*lilac* | *Lilac*-red | Red |
| Nylon | Light blue | Light blue | Light blue | Greenish blue | Almost colourless |

### 4. Effects of various colloid substrates on the colours of cobaltous salts

Howell & Jackson (1937*b*), using different glass and metal substrates with anhydrous cobalt chloride, showed that the nature of the substrate affected the absorption spectrum of the salt. Such effects may operate when different colloids are used, but in the presence of moisture the main variation arises from the different moisture-holding capacities of various colloids.

It has been mentioned that in filter paper impregnated with cobalt chloride, the blue → lilac → red change, with its range of easily matched reddish blues and bluish reds, is completed within the humidity range, 40–70% R.H. For the measurement of high humidities, it is an advantage to have this colour change higher on the humidity scale. Accordingly, the effects of a number of substrates other than filter paper were examined.

This involved essentially the same procedure as that described by Hatschek (1936) and by Kretovitch & Uschakova (1940), but whereas they used the blue colour of the cobalt chloride as an indication of the point at which no 'un-bound' water remained in colloids when gradually dried, the object of the present tests was to find which colloids retained the blue colour at high humidities.

A series of tests was made by thoroughly wetting

changes beyond certain limits—the colloid being chosen according to the humidity limit desired.

Alternatively, cobalt chloride may be used as an indicator of the moisture content of the colloid substrate, as it is in certain proprietary silica gels.

In another experiment, in which cobalt thiocyanate was used, a piece of cotton-wool was mercerized by boiling in potassium hydroxide solution. After washing and drying, it was found to be more hygroscopic than an untreated piece. Both were then impregnated with a solution of the cobalt salt. Similarly, a piece of viscose fabric of decreased hygroscopicity, due to treatment with formaldehyde, was impregnated together with an untreated sample.* The effects of the two treatments on the colour of the cobalt salt are illustrated by the observations in Table 6.

TABLE 6

| Substrate | Colour at 30% R.H. | Colour at 70% R.H. |
|---|---|---|
| Cotton-wool | — | Blue-lilac |
| Mercerized cotton-wool | — | Blue |
| Viscose fabric | Blue | — |
| Viscose fabric pretreated with formalin | Blue-lilac | — |

* The writer is indebted to Mr C. C. Mill, of the Printing and Allied Trades Research Association, who suggested the experiment and kindly supplied the two samples of viscose.

The opposite effects of mercerization and formaldehyde treatment on the hygroscopicity of cellulose are well known; the above results indicate that mercerization also increases the effect of cellulose on the colour of sorbed cobalt salts.

### 5. Effects of inorganic salts on the colours of cobaltous compounds

In order to render cobalt chloride paper more suitable for the measurement of high humidities, the possibility of adding other compounds to move the blue → lilac → red colour change higher up the humidity scale was investigated. Organic solvents were ruled out on account of their volatility, and the acids and alkalies because of their corrosive properties. Attention was concentrated on inorganic salts.

Those which have been reported to favour the development of the blue colour in cobalt chloride solutions include magnesium chloride (Hill & Howell, 1924; Bassett & Croucher, 1930; Howell & Jackson, 1936*a*), potassium thiocyanate (Howell & Jackson, 1937*a*) and the chlorides of lithium, rubidium, caesium, calcium, strontium, and thorium (see Bassett *et al.* 1937).

The writer found that salts such as magnesium chloride, calcium chloride, and aluminium chloride, although producing the blue colour when in high concentration in cobalt chloride solutions, did not maintain this effect on impregnated filter paper, because the water content rapidly increased owing to their hygroscopicity. Others, such as sodium chloride, potassium chloride, and dibasic sodium orthophosphate, raised the humidity level at which the papers retained the blue colour, but the effect was not great enough to be useful above 80% R.H.

The writer is indebted to Dr D. J. Crisp, with whom he discussed this work, and who pointed out the strong purple and blue colours attainable by mixing potassium thiocyanate with cobalt chloride solutions.* This effect was found to be maintained when the mixtures were held on filter paper, and potassium thiocyanate was adopted as suitable for the purpose in view.

A few later tests showed that other compounds would also be suitable: magnesium oxide has a marked effect in maintaining the blue colour, while sodium thiosulphate produces very striking results.

In the above tests, the aim was to produce an extension of the blue → red colour change as far as possible up the humidity scale, therefore the various salts were tested first at the highest concentrations possible. However, as the blue → red change is moved farther up the humidity scale, the method becomes less effective for use at lower humidities, since the various blues are comparatively difficult to differentiate. Thus the amount of salt to be added to the cobalt chloride solution depends on which part of the humidity range it is desired to measure most effectively. Using potassium thiocyanate with cobalt chloride, papers suitable for use over the range 40–100% R.H. may be prepared with a mixture of equal volumes of 50% w/v $CoCl_2.6H_2O$ and 25% w/v KCNS solutions.

Methods are available for shifting the blue → red change *down* the humidity range, if this is desired. Various salts, such as the chlorides of zinc, cadmium, mercury, tin, or antimony, promote the red colour when added to cobalt chloride solutions (for references to the original work, see Hill & Howell, 1924, and Bassett & Croucher, 1930). The effect is maintained on impregnated paper. A similar result can be achieved by mixing cobalt chloride with glycerol (Darrow, 1943).

### 6. Cobalt thiocyanate paper

A disadvantage of using potassium thiocyanate together with cobalt chloride is that two standard solutions must be prepared with a fairly high degree of accuracy, since the proportion of the two salts is critical. Further investigation led to the conclusion that a solution of cobalt thiocyanate was as effective as the mixture. Moreover, this involved the preparation of only one standard solution.

Accordingly, cobalt thiocyanate solutions were used for all further work, and the following sections describe the preparation and use of paper treated with this salt alone.

#### (*a*) *Relation between colour and humidity*

At humidities up to 55 or 60% R.H., paper strongly impregnated with cobalt thiocyanate presents a series of blue colours; the red component is scarcely discernible except in comparing the different blues among themselves. From 60 to 75% R.H., the colours may be classed as blue-lilac, from 75 to 90%, as red-lilac, from 90 to 100% as light red, although a faint blue component is detectable even at 95% R.H.

Occasionally, at low humidities, a brown form of cobalt thiocyanate appears on the paper. It is readily reconverted when the paper is exposed to higher humidities.

#### (*b*) *Selection of suitable paper*

The strength of the colours produced by cobalt thiocyanate made it possible to use a thinner material than filter paper. It was hoped that this might reduce the time required for equilibration. Also, the use of very thin pieces of material would reduce the dis-

* Howell & Jackson (1937*a*) showed that the addition of potassium thiocyanate to cobalt chloride produced changes analogous to those found when magnesium chloride or hydrochloric acid was added. The strength of the blue colour produced depended on the amount of potassium thiocyanate added, up to a concentration of 730 g./l. Their paper includes references to previous work.

turbing effect on humidities in small enclosed spaces. Further, it was considered advisable to eliminate the mineral matter (mainly chalk) present in filter paper.

In the selection of a suitable paper, the writer was assisted by valuable advice from Messrs J. A. S. Morrison and C. C. Mill, of the Printing and Allied Trades Research Association. A number of samples of thin paper were kindly provided by various firms. An examination of these led to the selection of 'Electrolytic "B" Condenser Tissue', a thin tissue of pure cotton fibre, without loading or size, produced by Brittain's Ltd., to whom the writer is indebted for a supply of the paper. The dry weight of this paper is about 229 mg./100 sq.cm.

*(c) Preparation of standard solution*

Various hydrates of cobalt thiocyanate are known, including the hemi-hydrate $Co(CNS)_2.\frac{1}{2}H_2O$. The material used was obtained in this form, as a dark brown crystalline mass containing a quantity of sorbed water.

Attempts were made to drive off the moisture with a view to preparing a standard solution gravimetrically. The material was heated in an oven at 110° C. for periods of $2\frac{3}{4}$, $5\frac{3}{4}$ and $11\frac{1}{2}$ hr.; the resulting figures for the moisture-content were 5·39, 5·48 and 6·25 % respectively of the total weight. When a small sample of the material was heated gently over a micro-burner, sulphurous fumes were evolved after 5·69 % of the original weight was driven off. When exposed over phosphorus pentoxide in a desiccator (without evacuation), the material lost weight continuously for at least 24 hr.; after 65 hr. the loss of weight was 5·69 %. Calculations based on the titration of a sample of the same material (see below) also indicated 5·69 % as the correct value for the moisture content.

It appears, therefore, that the sorbed moisture could be removed satisfactorily by drying over phosphorus pentoxide for at least 2 or 3 days, and that a standard solution could then be prepared gravimetrically. It is difficult to remove all the sorbed moisture by heating, without driving off some water of crystallization and causing decomposition of the thiocyanate.

The solution used in the preparation of the paper was standardized by titration. The writer is indebted to Mr F. P. W. Winteringham, of this Laboratory, for carrying out the standardization, and for his advice on the titration of the cobalt chloride solution (§ 3 (*a*)). His report is as follows: 'An approximately 3*N* solution was prepared by dissolving a calculated weight of crystalline cobaltous thiocyanate [$Co(CNS)_2.\frac{1}{2}H_2O$] in water. Exactly 1·000 ml. of this solution was made up to 250 ml. in a graduated flask, the solution being acidified with nitric acid. This solution was sufficiently colourless to enable the $Co(CNS)_2$ to be titrated directly with 0·1*N* $AgNO_3$, using ferric ammonium sulphate as an indicator. The original solution was thus found to contain 269 g. of pure cobaltous thiocyanate per litre of solution, the normality therefore being 3·073*N*. The estimated maximum error (titration errors etc.) was ±0·5 %.'

This solution was subsequently adjusted, using carefully checked glassware, by bringing the volume of 81·32 ml. aliquots up to 100 ml. with water; the normality (calculated) was then 2·498*N* and the solution was equivalent (by calculation) to one prepared by dissolving 23·00 g. of dry $Co(CNS)_2.\frac{1}{2}H_2O$ in water to a volume of 100 ml.

*(d) Impregnation of the paper*

The method for impregnating the paper with cobalt thiocyanate was slightly different from that described for the preparation of cobalt chloride paper. The tissue paper was cut to pieces of a convenient size and kept in a desiccator over calcium chloride. Each piece was immersed in a flat glass dish containing some of the standard solution (2·5*N*). The fragility of this paper when wet made its removal from the solution somewhat difficult. Therefore, the paper was laid on a sheet of glass or stout celluloid when immersing in the solution. After 1 min., during which the dish was gently rocked, the glass or celluloid support was lifted out with the paper adhering to it, and the whole was drained rapidly and lightly pressed by hand between two sheets of desiccator-dried No. 1 Whatman filter paper. The tissue paper could then easily be removed intact from its support, and then pressed between a second pair of dried filter papers with a squeegee.

Although this method was standardized as far as seemed possible, slight variations in the amounts of the deposits on different pieces of paper tended to occur. Possibly the concentration of the solution in the dish changed slightly as successive papers were immersed in it. The result was that some of the papers were slightly stronger in colour than others; this did not lead directly to errors in matching with the standards, but made the matching a little more difficult.

In an attempt to eliminate this variation, the writer also used an alternative method, which may be considered preferable. It eliminates the necessity of desiccating the paper before wetting, and does away with the mopping process afterwards. The paper is laid on a clean sheet of glass, the standard solution is put on directly in small drops from a capillary pipette, and the paper is left to dry on the glass. By dabbing the paper gently with the tip of the pipette, as uniformly as possible over its surface, the solution can be distributed so as to produce a paper as uniform in colour as one prepared by the earlier method. (In both cases there is a close pattern of denser and lighter colour due to the inevitable unevenness in texture of the paper.) The amount of solution required to produce papers of the same depth of colour as those prepared by the first method is 0·26 ml./100 sq.cm. of paper.

It is convenient to cut the paper, when dry, into pieces somewhat smaller than 1 × 2 cm., ready for use.

(*e*) *Preparation and use of standards*

Standards are prepared by the method referred to in § 3 (*b*). Each piece of cobalt thiocyanate paper is exposed for 2 hr. in a bottle over a potassium hydroxide solution of appropriate strength (Buxton & Mellanby, 1934). It is important that these solutions be kept at constant temperature, to avoid humidity variations in the bottles. The rubber stopper of each bottle is provided with a metal hook on the underside, so that the paper can be suspended over the solution by means of a thin wire. At the end of 2 hr., the stopper is lifted from the bottle and the paper rapidly immersed in liquid paraffin in a wide-mouthed vial. The paper is doubled over (to intensify the colour) before being mounted in liquid paraffin between the white opal glass and the clear glass. The sizes used are approximately: paper 8·5 × 18 mm. (before doubling over), opal glass 25 × 30 mm., clear glass 20 × 25·5 mm. The cover-glasses are cut from colourless microscope slides. The doubled paper is orientated symmetrically on the opal glass, then the clear glass is lowered over it carefully from one side to avoid including air bubbles. A standard weight is placed on the middle of the cover-glass. A convenient form of weight for the purpose is a vial containing about 50 g. of mercury.

The excess oil is removed from the edges of the glass with blotting paper; enough should be removed so that the oil just begins to recede inwards away from the edge of the cover-glass. A stiff solution of sodium silicate is then applied with the tip of a thin glass rod along the angle left at the edges of the opal glass by the smaller cover-glass. It is left to stand for at least 12 hr., then the weight is removed, any loose oil wiped away, and another application of sodium silicate given. Then the weight is replaced for another 12 hr. period. The standard, then ready for use, consists of the paper held between the two pieces of glass, surrounded on all sides by the oil, which extends to the sealed-up edges.

Difficulties arise if the sodium silicate solution is not sufficiently viscous. Liquid glues may be used instead of sodium silicate, and applied in the same way, but the silicate has the advantage of being colourless.

Standards prepared in this way have not yet been kept for long periods, but the colours of sets prepared 4 months ago appear identical with those of fresh papers.

Occasionally, alkali from the sodium silicate penetrates the oil and affects the paper, if the adhesive has been allowed to pass in under the cover-glass—an accident which is less likely to occur when the thinner paper is used.

The standards may be conveniently mounted in order on boards provided with flanges to grip the edges of the glass. It is important that the mounting should not prevent close examination of the papers in full light. The number of standards in a set is determined by the range of humidity to be covered and by the minimum convenient size of the colour-increments. Intervals of 5 % R.H. have been found convenient, and since simple cobalt thiocyanate papers are adapted for work at humidities above 50 %, only the 50–100 % R.H. range is covered in this way; papers covering the 0–50 % R.H. range, spaced at intervals of not less than 10 %, may be useful for rough estimations of low humidities.

In matching test papers against the standards, account is taken of both red and blue components of the colours. If the test paper does not match any of the standards exactly, it is possible to estimate the correct value to the nearest 1 % by interpolation. As with colour examination in general, it is more effective to glance briefly at the papers several times than to stare at them continuously, which leads to fatigue and inaccuracy.

(*f*) *Time required to reach equilibrium*

This depends on the temperature and humidity at which the papers are exposed. The following statements, summarizing the results of many tests in which comparisons were made between the colours of papers exposed for various periods, illustrate this double relationship:

At 7° C., equilibration is completed within 30 min. or less up to about 85 % R.H., whereas at higher humidities, up to 2 hr. is required.

At 20° C., equilibration is completed within 10 min. up to about 70 % R.H., within 15 min. up to about 90 % R.H., within 60 min. up to 94 % R.H., while at higher humidities up to 2 hr. may be required.

At 30° C., equilibration is completed within 30 min. or less up to about 96 % R.H., above which a somewhat longer period is required.

The paper used in these tests was in a fairly dry condition (strong blue in colour, as it would normally be kept), at the beginning of the exposure period. It will be seen that exposure for 2 hr. is necessary to cover all the conditions of temperature and humidity, but a period of 30 min. is sufficient under all conditions excepting those of very high humidity (the critical humidity level depending on the temperature). At low humidities, exposure periods considerably less than 10 min. are sufficient, but the papers under discussion are not well adapted for use at low humidities.

(*g*) *Effects of temperature*

As in the case of the cobalt chloride paper, the direct effects of temperature on the colour may be neglected, when papers are being compared with standard papers mounted in oil, so long as both are at about the same temperature during the comparison.

The indirect effects, due to the influence of temperature on the amount of moisture held by the paper-salt complex at a given humidity, are more marked at low temperatures than in the case of cobalt chloride paper. Thus, at 7° C., papers exposed to 50% R.H. read 3·5% R.H. too high; to 60%, 3% too high; to 70%, 3% too high; to 80%, 2% too high; and to 90%, 2% too high.

As shown in § 6 (*f*), the time necessary for the paper to reach a steady colour at a given humidity is influenced by temperature; e.g., papers become equilibrated at 94% R.H. (as judged by colour) within 30 min. at 30° C., while about 1 hr. is required at 20° C.

### (*h*) *Accuracy of the method, and correction factors*

It will be seen from the above that the main causes of error are incomplete equilibration of papers, the indirect effects of temperature, and inaccurate matching of papers. As with cobalt chloride papers, no appreciable hysteresis effects were detected.

When even shorter exposure times are allowed, errors arise at high humidities, unless corrections

TABLE 8

| Humidity values indicated by papers | Exposure times | | | | |
|---|---|---|---|---|---|
| | 10 min. | 15 min. | 30 min. | 1 hr. | 2 hr. or more |
| % R.H. | % R.H. | % R.H. | % R.H. | % R.H. | % R.H. |
| 75 | Add 1 | 0 | 0 | 0 | 0 |
| 80 | Add 2 | 0 | 0 | 0 | 0 |
| 85 | Add 3 | Add 1·5 | 0 | 0 | 0 |
| 90 | Add 5·5 | Add 2 | 0 | 0 | 0 |
| 92 | Add 6·5 | Add 2·5 | Add 1 | 0 | 0 |
| 93 | Add 7 | Add 3 | Add 1 | 0 | 0 |
| 95 | — | Add 5 | Add 2 | Add 0·5 | 0 |
| 96 | — | — | Add 2·5 | Add 1 | 0 |
| 97 | — | — | Add 3 | Add 1·5 | 0 |
| 98 | — | — | — | Add 2 | 0 |

are applied. Table 8 gives such corrections for use when the temperature is between 15 and 25° C. (59–77° F.).

TABLE 7

| Humidity values indicated by papers | Temperature | | | | |
|---|---|---|---|---|---|
| | 0–5° C. (32–41° F.) | 5–10° C. (41–50° F.) | 10–15° C. (50–59° F.) | 15–25° C. (59–77° F.) | Above 25° C. (above 77° F.) |
| % R.H. | % R.H. | % R.H. | % R.H. | % R.H. | % R.H. |
| 50–75 | Deduct 4 | Deduct 3 | Deduct 2 | — | — |
| 75–85 | Deduct 3 | Deduct 2 | Deduct 1 | — | — |
| About 92 | Add 2 | Add 2 | Add 2 | Add 1 | — |
| About 95 | Add 4 | Add 4 | Add 4 | Add 2 | — |
| About 97 | — | — | — | Add 3 | Add 1 |

Assuming fairly good colour vision, it is estimated that, if 30 min. exposure time is allowed, and the temperature is between 5 and 33° C., errors from these sources may amount to as much as ± 5% R.H. under certain conditions. But if the conditions under which the chief errors arise are avoided, the method can be used with considerably greater accuracy. All appreciable equilibration errors can be avoided by allowing 2 hr. exposure time instead of 30 min., whenever the humidity is over 80% R.H.

At low temperatures, more moisture is taken up, and the red component increases at the expense of the blue, thus causing the papers to indicate *too high* a humidity. But the effect of low temperature on the equilibration of the papers is to retard it, i.e. to cause them to indicate *too low* a humidity (assuming the papers are fairly dry at the beginning of the exposure period). Advantage may be taken of these two opposing tendencies by restricting the exposure time at low temperatures to 30 min. when the humidity is over 85% R.H. Table 7 gives approximate corrections for low temperatures; the corrections for humidities above 85% apply only when a 30 min. exposure time is allowed.

Unlike cobalt chloride papers, thiocyanate papers appear not to change in colour even after several days' exposure at high humidities such as 95% R.H. However, the precautions against exposure to a saturated atmosphere, and against actual wetting, are equally necessary with both.

If particles of flour are allowed to adhere to papers exposed to high humidities, the flour takes up some of the thiocyanate and assumes a bluer colour than the paper, thereby causing errors. The writer is indebted to Mr G. T. Jefferson and Dr J. H. Fraser, of the Ministry of Food Infestation Branch, who pointed out this fact. Extensive contamination of the papers by foreign materials should in general be prevented.

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*List of Common British Plant Diseases.* Compiled by the Plant Pathology Committee of the British Mycological Society. Pp. 61. Cambridge University Press. 1944. 5*s.* 0*d.*

This useful publication has now reached its third edition. The title has been changed from the original *List of Common Names of British Plant Diseases*, but the present title still does not indicate that, in addition to the names of diseases, the book contains an equally useful and authoritative list of the causative agents of the diseases.

There is an informative 'Introduction' and the text has been revised and rearranged in a much more convenient form. Thus, instead of the host plants being grouped as cereals, pasture and forage crops, vegetables, root crops, etc., they are now arranged alphabetically according to the name by which they are best known, and the names of tree diseases and their causative agents have been included. A further improvement is the grouping of the causative agents of the diseases of each host in order of fungi, bacteria, viruses, non-parasitic, and the alphabetical arrangement of the several fungal and bacterial diseases according to the scientific names of the parasites. Owing to the alphabetical arrangement of hosts the 'Index of Hosts' of previous editions becomes superfluous and is omitted; the 'List of Authors' Names and Abbreviations' has been extended; and there are new indexes of 'Foreign Common Names' (including Russian) and of 'Latin Names'.

As one would expect of a Committee of the British Mycological Society, the names of fungi are stated, and rightly so, with punctilious regard for the *International Rules of Botanical Nomenclature*. I find the Committee's rigid adherence to the *Rules* a little over-conservative and burdensome in everyday practice, and I much prefer the common American usage and welcome the lead given to plant pathologists in this country by the Imperial Institute of Mycology and Ainsworth & Bisby's recent *Dictionary of the Fungi*. The Committee, in accordance with the Rule adopted at the International Congress for Microbiology, 1936, have, of course, adopted small letters for specific names of bacteria. In the *List* even the common names of diseases are capitalized: thus, brown foot rot and ear blight of wheat becomes 'Brown Foot Rot and Ear Blight' of wheat, which seems to me excessive, especially in view of the fact that in textbooks and journals common names may often be repeated several times on a page. In the *Annals* we have long adopted small letters for all common names of parasites, diseases and host plants, indeed for all names used colloquially, and I hope that this practice will be maintained in spite of our general acceptance of the *List* as a guide to standardization.

The Committee's treatment of viruses seems perhaps over-timid. The 'Introduction' states: 'Several methods of naming viruses have been proposed in recent years, but it is thought desirable to avoid recommending any one method until a decision is reached by the International Committee appointed for the purpose at the 5th International Botanical Congress held in Cambridge in 1930.' In the *List*, names of viruses are included, the alphabetical method being adopted, so that actually there is recommendation. But the *List* gives no indication that, for example, 'Potato virus *X*' stated to be the causative agent of 'Leaf Drop Streak' of potato is the same as Kenneth Smith's '*Solanum* virus 2', Holmes's '*Marmor cucumeris* var. *upsilon*', etc. It would have been useful if, as a tentative measure, these other names had been included, perhaps as synonyms, for they have been widely adopted in both scientific journals and textbooks. We have already waited some 15 years for the International Committee's Report on virus nomenclature and, with the world and virus diseases in their present state, another similar period might seem an optimistic estimate; meanwhile, chaos becomes worse confounded.

However, as a guide to standard common names and accurate scientific nomenclature the recommendations of the *List* should undoubtedly be accepted. The science of phytopathology is, indeed, greatly in debt to the Plant Pathology Committee of the British Mycological Society for the skill and acumen they have shown in this compilation and for the arduous and time-consuming labour it must have involved.

WILLIAM B. BRIERLEY

*Plant Viruses and Virus Diseases.* By F. C. Bawden. 2nd edition. Pp. xi+294. Waltham, Mass.: Chronica Botanica Co. (London: Wm. Dawson and Sons, Ltd.). 1943. $4.75.

The first edition of this book (noted in the *Annals*, 1940, vol. **27**, p. 576) came to an untimely end with the invasion of the Netherlands. The only compensation was that it gave the author an opportunity to prepare a new edition which, as the Publishers are now established in America, has been produced in very pleasing fashion. In general plan the book remains unaltered but it is arranged in sixteen instead of fifteen chapters, has increased slightly in length, every chapter has been revised and several completely rewritten, and new tables, illustrations and references have been inserted. As in the first edition emphasis is directed throughout to physical, biochemical and serological aspects as is perhaps natural of a writer who has himself taken a leading part in advancing these avenues of knowledge. The book is a first-class survey of modern plant-virus research which, in so difficult a field, is a noteworthy achievement.

It has been becoming increasingly obvious during the last decade or so that the time-honoured departmentalization of science is obsolete and that scientific research workers must be competent to move freely across the compartments of science still imposed by its teaching organization. In no field of work is this clearer than in virology and in no book that I have read of recent years are these developments so clearly indicated. In plant virology abstruse problems of morphology, physiology and cytology integrate with equally abstruse problems of medical and veterinary pathology, entomology and genetics; they are examined by means of elegant techniques of microscopy, serology, biochemistry, physical chemistry and pure physics; whilst hovering over all is the controlling hand of statistics. It is an astonishing picture that remains in one's mind after reading this book, one utterly different from that of 20–25 years ago when it was generally accepted that plant viruses and virus diseases were the domain of the botanically trained 'mycologist'. It seems to me quite clear that human, animal and plant virology are merely aspects of one scientific discipline, a discipline which has come of age, and which must be welcomed as an independent and autonomous science in its own right.

Virology is a comparatively newly discovered and relatively undeveloped continent and Mr Bawden's work has given him an assured place among the pioneer explorers.

WILLIAM B. BRIERLEY

# Laws of the Association of Applied Biologists

I. The Association shall be called "The Association of Applied Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two Members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the Members present and voting shall be cast in favour of the candidate.

V. All Ordinary Members on first election shall pay an entrance fee of half-a-guinea. Ordinary Members shall pay an annual subscription of twenty-five shillings, due on 1 January of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every Member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a Member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A Member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary Members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each Member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no Member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new Member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any Member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publication and shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, two Secretaries, an Editor and an Assistant Editor of the Annals, and twelve Ordinary Members, four of whom shall retire each year and shall not be eligible for re-election within one year. The retiring President may be invited to serve as an additional member of Council and as a Vice-President for a period of two years. One other member of Council shall be nominated by the President to act as a Vice-President.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six Members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any Member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a Member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any Member two-thirds of the votes of the Members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two ordinary members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the

Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorize their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a Council meeting not less than one month prior to the Annual General Meeting, the Council shall nominate a President, a Treasurer, two Secretaries, and an Editor and an Assistant Editor of the Annals. At least three weeks before this Council meeting a notice shall be sent to each Member resident in the British Isles giving the names of the four retiring members of Council and asking for nominations, duly seconded, to fill the four vacancies. These nominations shall be included with any put forward by the Council. The list of nominations for Officers and ordinary members of Council shall be sent to all Members resident in the British Isles, at least two weeks before the date of the Annual General Meeting.

The election of Officers and ordinary members of Council shall be conducted in the following manner: At the Annual General Meeting the list of nominations for Officers and for the Council vacancies shall be read. If the number of persons proposed does not exceed the number to be elected the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If there are more names than positions to be filled a ballot shall be taken. The Chairman shall appoint from among the Members present two persons, not candidates for election, to act as Scrutineers. Each Member voting shall hand in person to one of the scrutineers a copy of the list on which has been indicated the names of those candidates whom the Member voting desires to support. The Scrutineers shall reject any ballot paper which supports candidates in excess of the number to be elected. They shall report to the Chairman of the meeting the number of votes cast for each candidate and the Chairman, before the close of the meeting, shall announce the names of those elected. In the case of an equality of votes for any candidates, the Chairman of the meeting shall choose between them before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each Member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall, unless otherwise decided by the Council, be held on the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this Annual General Meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten Members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.

*Note*

The following resolutions have been passed by the Council of the Association:

(1) That manuscripts reporting investigations dealing primarily with proprietary substances of which the composition or nature is not specified in such a way that the investigations can be repeated by other workers be not eligible for publication in the *Annals of Applied Biology*.

(2) That the mention of proprietary substances without specification of nature or composition be permissible in a manuscript provided that the particular substances are well known or standard and are only incidental in the work or contributory or subsidiary to the main theme or are used for purposes of control or comparative experiment.